Analysis of the spatial and temporal dynamics of herbicide resistance to ACCase- and ALS-Inhibitors in *Alopecurus myosuroides* Huds. and their causes

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Abbreviations

A_{max} maximum number of seeds of *A. myosuroides* per plant in the absence of herbicide application and competition

D_{max} maximum number of *A. myosuroides* plants per sqm

ANOVA Analysis of Variance

AVESA *Avena sativa* L.

BEAVU *Beta vulgaris* L.

BRSNS *Brassica napus* L. (summer)

BRSNW *Brassica napus* L. (winter)

CA Cellular Automaton

d Day

DEN Phenylpyrazoline, family of ACCase-inhibitor herbicides

DIM Cyclohexanediones, family of ACCase-inhibitor herbicides

EMR Enhanced metabolic Resistance

FK Field Capacity

FOP Aryloxyphenoxypropionates, family of ACCase-inhibitor herbicides

GRUND Soil Depth

HORVS *Hordeum vulgare* L. (summer)

HORVW *Hordeum vulgare* L. (winter)

I developing resistance

IWM Integrated weed management

KAK Cation Exchange Capacity

MatPfl total number of plants at the end of the season

MoA Mode of Action

MutRate Mutation Rate

paG Germination Rate

PC Principal Component

PCA Principal Component Analysis

PIBSA *Pisum sativum* L.

pS surival rate of seeds in the soil

R Resistant

ResistanceFreqA Initial Frequency of A in the population

ResistanceFreqB Initial Frequency of B in the population

ResistanceFreqC Initial Frequency of C in the population

S Sensitive

SeedPlant number of seeds produced per plant in a given cell

SOM Soil Organic Matter

TRZAS *Triticum aestivum* L. (summer)

TRZAW *Triticum aestivum* L. (winter)

TSR target site resistance

TTLWI Triticale (*Triticum aestivum* L. x *Secale cereale* L.)

UFK Usable Field Capacity

ZEAMX *Zea mays* L.

Nomenclature

BBCH Growth stage according to the BBCH scale

Fst Fixation index, analysis of genetic structure to differentiate between populations, Weir and Cockerham (1984)

ha Hectares, 1ha=10000m^2

km Kilometers, 1km=1000m

Resistance Factor Difference in e.g. ED^{50} between resistance and sensitive Biotype

sqm Square Meter

X An Amino acid substitution that at a given location that is not defined due to multiple possible substitutions.

Chapter 1

Introduction and Aim of the Study

1.1 Introduction

Weed management has always been among the challenges in agriculture, with definitions of the term "weed" being widely available. Baker (1974) characterizes a plant as a weed if "its populations grow entirely or predominantly in situations markedly disturbed by man" without being a crop. Competition with the crop for resources is therefore the main reason for weeds being undesired. Oerke (2006) describes yield losses due to weeds as the highest among weeds, insects and pathogens, highlighting the importance of their control in ensuring high yields. Weed management therefore describes long and short term human interventions to support the crop and sustain yields. While before the Second World War this was dominated by mechanical weeding, the introduction of herbicides has changed the game. High efficacy at a comparably low price has paved the road for the success of chemical control measures. However, increasing numbers of reported cases of herbicide resistance not only limit the options for farmers tackling these weeds, but also reduce their yields. Therefore the task of the weed scientist is, among other things, the development of applications to reduce weed pressure and the yield losses it causes. This task is now being made more challenging, looking at the lower number of available chemical tools and the increasing rate of herbicide resistance due to simplified crop rotations and increasing weed seed banks. In a recent critique Ward et al. (2014) pointed out that this challenge is largely not accepted within the weed science community as "the utility of novel research technologies in agricultural weed science is too often undermined by the substitution of descriptive studies and mere fact collecting in place of the pursuit of more rigorous questions". Within this work, a deeper understanding of the temporal and spatial dynamics of herbicide resistance in a defined environment shall be developed using *Alopecurus mysoduroides* as a model plant for an outcrossing species. The connection of the findings with both environmental and management factors shall be established. Lastly, the ideas and discoveries of the previous two points will be used to develop a basis for an application that enables the farmer to predict the resistance risk on individual fields to proactively manage the development of resistance instead of acting after a problem has occurred in the field causing yield loss.

1.2 Literature Review

1.2.1 Alopecurus myosuroides

Biology

Alopecurus myosuroides Huds. is an annual diploid species with 2n=14 chromosomes belonging to the *Poaceae* family (Kemmer et al., 1980). In earlier articles it is referred to as *Alopecurus agrestis* as in e.g. Koch (1846); Fruwirth (1908). While Fruwirth (1908) described *A. myosuroides* as very similar in appearance to *A. pratensis*, with only minor morphological differences in the ear, it was found that *A.pratensis* has twice the number of chromosomes (2n=28)(Kemmer et al., 1980). *A. myosuroides* is an out-crossing species as is described by Chauvel and Gasquez (1994). Self fertilization occurs rarely and produces only a few viable seeds (Wöhrmann, 1960; Chauvel and Gasquez, 1994). One reason for self incompatibility is a delay of anther emergence 1-5 days after the main pollen shed on the same ear. This leads to inbreeding on only those plants with a large number of tillers (Menck, 1968).

Distribution, Occurrence & Spread

A. myosuroides mostly occurs in central and northern Europe, where it has become a noxious weed (Moss et al., 2007). It has however also been reported in other countries around the Mediterranean sea, from which it most likely originated. Reports of *A. myosuroides* in some Asian countries also exist (Kemmer et al., 1980). The weed has also been found in North America and New Zealand (Kemmer et al., 1980). Ellenberg (1979) puts *A. myosuroides* as indicator for mildly warm climates (Temp=6) and categorizes it as occurring in Central and Western Europe (K=3). For Germany Brückner (1958) describes two main areas of distribution. One in Northern Germany including parts of Westphalia and Hesse and the other in Southern Germany in the state of Baden-Württemberg. Menck (1968) studied the distribution of *A. myosuroides* in the state of Schleswig-Holstein, discovering regional differences in the abundance of the weed that he attributed to soil conditions in combination with the crop rotation used. In Southern Germany Fruwirth (1908) studied the abundance of the weed for many parts of the State of Baden-Württemberg. For the area of Hohenlohe, where this study was conducted, he found differences in abundance of the weed at different sampling locations. He concluded that the abundance of *A. myosuroides* was not attributed primarily to the crop

rotation but rather other factors that were not defined further. The differences in occurrence between neighboring fields lead frequently to the question of spread regarding *A. myosuroides* .

While pollen flow was observed in rare cases under certain conditions it is believed that the exchange of pollen occurs only within a field (Menck, 1968; Chauvel and Gasquez, 1994). Older literature also reports the distribution of seeds (via harvest and tillage equipment) which was observed after the Second World War (Brückner, 1958; Menck, 1968). Personal communication with farmers also brings up these particular issues as custom harvesters gain popularity. Anecdotal evidence of heavy infestation levels suggesting that weeds spread by harvest equipment are reported frequently. However, at wheat harvest most of the seeds are shed.

Genetic variation

Genetic variation is very high and very little differentiation between populations from different origins occurs (Chauvel and Gasquez, 1994; Menchari et al., 2007b). Menchari et al. (2007b) did not find evidence for a "demographic bottleneck" meaning that the introduction of herbicides but also other very rapid changes in the environment of the different populations assessed did not effect the populations genetic diversity. Furthermore, these authors found that several populations within one field (subpopulations) were genetically different from each other and were also found in other geographical areas. However, Chauvel and Gasquez (1994) discovered by calculating the mean heterozygosity that panmixa can be assumed for *A. myosuroides* . Exchanging genes between the subpopulations will consequently result in plants with new properties. Menck (1968) described such varieties and their morphological traits, describing a wide range of different phenotypes. However, while he found some of the morphological traits to be heritable, most of them were not and are probably expressed as a result of environmental effects given that some were found more frequently in certain locations (Ecotype).

Environmental Requirements

Soils with constant water supply as observed in heavier soils are better suited to carry high levels of *A. myosuroides* (Menck, 1968). It was also reported by Menck (1968) that the constant water supply is especially important for good germination of *A. myosuroides* . Furthermore, in an experimental setup he found that *A. myosuroides* loses its competitive advantage over the wheat crop where waterlogging is reduced. Brückner (1958) also

reported that fields with high ground water levels and insufficient or no drainage experience higher *A. myosuroides* levels Menck (1968). While *Apera spica-venti* prefers sandy to loamy soils with lower nutrient supply and lower pH-values, *A. myosuroides* prefers higher pH-values with high nutrient levels (Menck, 1968; Ellenberg, 1979). The positive influence of increased loam and clay levels on abundance and yield of *A. myosuroides* is probably the results of the capability of these soils to store larger volumes of water. Ellenberg (1979) classifies *A. myosuroides* as having a moderate light demand.

Growth and Development

Seeds of *A. myosuroides* can undergo two different dormancy processes. The first, primary dormancy, is mostly an after-ripening process (Samenreife), the second can last longer, preventing seeds from germinating the subsequent autumn(Menck, 1968). It was found that primary dormancy can be interrupted by higher temperatures, with colder temperatures promoting secondary dormancy.

Germination of *A. myosuroides* occurs mainly in autumn when optimal germination temperatures range between 10-15℃ while germination in general is possible between 2-35℃ (Menck, 1968). However, others report that germination already begins to occur at 0℃ with an optimum at 8℃ (Colbach et al., 2002). Despite the temperature requirements there are other factors reported in the literature that influence the germination process. Menck (1968); Colbach et al. (2002) reported that light also significantly improves the germination for both freshly produced seeds and seeds that have been buried in the soil longer. A short light induction of 1/1000sec already showeded good effects, with germination rates increasing with longer light periods (Menck, 1968). This partly explains why germination of *A. myosuroides* occurs mainly between 2-5cm where 90% of the seeds germinate (Naylor, 1970). This was confirmed by Menck (1968) in a deeper analysis, however he found successfully germinated and developed plants of up to 15cm depth. While Menck (1968) also found that oxygen increased the germination process and carbon dioxide inhibited it, the effects were smaller than with the other factors described . Seeds are exposed to changes in both oxygen level and light intensity during tillage, thus having an important influence on *A. myosuroides* germination rate. Older recommendations based on these findings promote non-inversive tillage to achieve high germination rates when primary dormancy is low (Menck, 1968). An extensive review by Lutman et al. (2013) on the other hand promotes ploughing as a successful means

of reducing *A. myosuroides* plants in the subsequent crop based on an analysis of several individual studies. The best combination of all of these factors occurs in autumn in western Europe, where germination for *A. myosuroides* is at its highest(Menck, 1968). Of the plants occurring in the subsequent crop, Naylor et al. (1972) found that 60-70% were from the previous year. Colbach et al. (2002) also reported that 38-70% of seeds are ready to germinate in the subsequent season. This larger variation was part of a deeper investigation by Swain et al. (2006) which found that the rate of seed germination in the subsequent season is linked to the climatic conditions at maturation. Under warm and dry conditions more seeds germinate in subsequent crops than do with cool and wet conditions. Temperature seems to have the biggest effect, however(Swain et al., 2006). This is in contrast to data reported by Menck (1968) who linked primary dormancy to the wetness of the location of the mother plant.

The development of successfully germinated plants is mostly synchronous with the development of the winter wheat crop, being slightly behind in autumn and compensating for this in spring (Menck, 1968). Seedlings were found to not survive frost while tillered plants, which are found in early sown winter wheat, survive down to -25℃ (Naylor et al., 1972). The period between the plant's shooting and the emergence of the ear is typically not more than 10–12 days with the plant flowering in June/July (Brückner, 1958). Seed production depends on several factors and the final number of viable seeds depends on the number of ears/plant, the number of seeds/ear and the number of successfully pollinated flowers. The number of ears per plant in a winter crop is usually 3–5, with only 1–2 in a summer crop Kemmer et al. (1980). These numbers can vary significantly as Menck (1968) reported plants with considerably more ears under favorable conditions.

Seed production per individual ear is usually around 30 (viable) seeds with up to 375 seeds also found (Menck, 1968). This leads to a total number of seeds per plant varying from 20-20000 (Menck, 1968), 87-3625 Brückner (1958) for *A. myosuroides* and 150-200 with a maximum of 600 for *A.pratensis* (Fruwirth, 1908). However, these authors did not assess the number of viable seeds out of the total number produced. On the ears formed from secondary nodes late in the season Moss (1983) reports that they are not important in significantly contributing seeds to the next generation. The reason for this can be found in the pollination of the plant. Moss (1983) found that the viability of seeds that shed the earliest and the latest is low, as the pollen cloud at this time is not yet well established and many flowers do not get well pollinated. This might also be a problem for *A. myosuroides* plants growing in dense row crops at low population sizes such as in

Zea mays.

Control

Chemical Control of *A. myosuroides* is still the most widely applied control technique and is reflected indirectly by the large number of publications seeking to identify the potential non-chemical alternatives as a response to resistance (Moss et al., 2007; Lutman et al., 2013). Among the most widely used ACCase and ALS-inhibitors numerous resistant populations were detected already (see Heap (2015) and chapter 1.2.3). However, there are other Modes of Action (MoA) available on the market to provide alternatives such as pre-emergent compounds (HRAC classification K1, K3, N), photosystem II inhibitors and glyphosate. ACCase and ALS-inhibitors were still the most favorable due to their selectivity and post emergent nature, enabling the farmer to control autumn and spring germinated plants with one application. This solution was widely applied for economic reasons until the occurrence of herbicide resistant *A. myosuroides* populations (Moss et al., 2007). Solutions based on herbicides will only face more pressure because of the EU legislation to reduce the pesticide impact, requiring more integrated weed management solution, including chemical and non-chemical solutions.

Non Chemical Control of *A. myosuroides* involves the crop rotation, tillage, delayed seeding and competitive varieties reviewed by Lutman et al. (2013). Generally a shift from a winter cereal-dominated crop rotation to more summer crops has positive effects in terms of lowering the number of germinated *A. myosuroides* plants in the crop (Fruwirth, 1908; Brückner, 1958; Menck, 1968; Jacobs, 1973; Kruecken, 1975; Lutman et al., 2013). While Menck (1968) reports a reduction of *A. myosuroides* when the portion of winter cereals is below 60-65% Jacobs (1973) reports a reduction with a 50% share of summer crop in the rotation. Lutman et al. (2013) reported that seeding wheat in spring as opposed to autumn reduced the number of plants by 88% if no other measures were taken. Mouldboard ploughing prior to sowing winter wheat conferred a reduction of emerged plants of 69% as reported in Lutman et al. (2013). However, Fruwirth (1908) previously reported that ploughing alone is not sufficient if *A. myosuroides* densities are high. Delaying the seeding by one month reduced *A. myosuroides* emergence by 50% (Lutman et al., 2013). Melander (1995) described the positive effect of later sown crops, with not only reduced numbers of *A. myosuroides* emerging but also a reduced formation of tillers formed within later sown crops, leading to lower number of seeds. However, sowing crops later increases the risk of uneven, less competitive crop stands with patches

in which *A. myosuroides* can develop very well and form large plants producing high amounts of seeds, as Brückner (1958) pointed out. Lastly, the use of more competitive varieties was reported to have positive effects in reducing the number of *A. myosuroides* by 22% as Lutman et al. (2013) reported. The effects are similar to the previously mentioned effect of delayed seeding as it reduces the number of ears while the total plant number is not effected. All non chemical control measures lack consistent efficacy compared to herbicides, making it difficult to convince farmers to use them (Moss, 2015). Assessing different combinations of these factors to see which combinations are synergistic and which are antagonistic is also very difficult as the success of these factors also depends on weather and soil conditions. With this Case Study we will therefore try to identify systems rather than individual factors that lead to to a successful control of *A. myosuroides* and follow the principals of integrated weed management.

1.2.2 ALS- and ACCase-Inhibitors

ACCase-Inhibitors are among the most widely used MoA against *A. myosuroides* and were introduced to the market over 30 years ago. They influence the first step in the synthesis of very long chain fatty acids by inhibiting the acetyl coenzyme A carboxylase (reviewed by Délye (2005)). This results in a failure to produce new cell membranes. There are three chemical classes within this family, theAryloxyphenoxypropionates (FOP), the Cycloheanediones (DIM) and Phenylpyrazoline (DEN).Broadleaf plants are naturally tolerant to ACCase-inhibitors as they have a different molecular structure of the enzyme, preventing the herbicide from binding (reviewed by Délye (2005)). This makes them ideal candidates for selective grass weed control in broadleaf crops, though they are also widely applied in cereals. Here the selectivity is gained by rapid metabolization of the compound in the crop sometimes triggered by safeners. However, at the field rate this does only apply for the FOPs and DENs for the currently registered ACCase-inhibitors against *A. myosuroides* . This group of herbicides is commonly referred to as group A herbicides according to the HRAC Mode of Action (MoA) classification.

ALS-Inhibitors ALS-inhibitors work by inhibiting the acetohydroxyacid synthase (reviewed by Tranel and Wright (2002)). This prevents the production of the branched chain amino acid isoleucine, leucine and valine. Commonly they are also referred to as Group B herbicides based on MoA classification by HRAC. It is a chemically diverse group active against both dicot and monocot weeds in many different crops. There are five chemical structures belonging to this group, the Sulfonylureas, the Imidazolinones,

the Triazolopyrimidines, Sulfonylamino-carbonyl-triazolinones and the Pyrimidinyl (thio) benzoates. Among these groups only the Sulfonylureas and the Triazolopyrimidines play an important role in the selective control of grasses in cereals crops. As for ACCase-inhibitors the selectivity in crops is achieved by differences in metabolism either occurring naturally or supported by safeners.

1.2.3 Herbicide Resistance

> "Herbicide resistance is the inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type. In a plant, resistance may be naturally occurring or induced by such techniques as genetic engineering or selection of variants produced by tissue culture or mutagenesis.[1] (WeedTechnology, 1998)"

Mechanisms of resistance

Extensive reviews of both occurrence and mechanisms of herbicide resistance can be found in Powles and Yu (2010) and Heap (2014) among others. Herbicide resistance can thereby be classified into several different mechanisms according to Heap (2014). Mechanisms reported for *A. myosuroides* are:

- Target site resistance where a mutation at the site of action prevents the binding of the molecule. This has been frequently reported to occur in *A. myosuroides* (Délye et al., 2005; Menchari et al., 2007b; Knight, 2016).
- Enhanced metabolism uses enzymes to detoxify the herbicide before reaching the site of action. Various examples for *A. myosuroides* also exist here (Délye et al., 2010b; Beffa et al., 2012; Knight, 2016).

There are however other mechanisms that have been described for other weeds and MoA depart from the two MoA being the focus of this work.

- Decreased absorption and translocation, preventing the herbicide from reaching its site of action (Goggin et al., 2016).
- Overexpression of the site of action, enhancing the gene copy number (Gaines et al., 2010). This theoretically requires a higher amount of herbicide to be sprayed, though the amount required usually exceeds the dose rate registered.

[1] In crops this is commonly referred to as tolerance

- Sequestration of the herbicides in cell walls or the vacuole where the amount of herbicide reaching the target is little or none, causing no damage (Lasat et al., 1997).

Target Site Resistance can be very versatile as multiple mutations coding for different mutations can comprise resistance to one MoA. Target site resistance (TSR) is usually restricted to one MoA with cross resistance to other chemical classes effective on the same MoA but not the other MoA. A fitness penalty might occur if the mutation impairs proper binding of the substrate therefore hindering biological processes within the plant (Powles and Yu, 2010). The Resistance Factor can be higher compared to Enhanced metabolic Resistance (EMR).

Enhanced metabolism on the other hand describes all mechanisms involved in the rapid detoxification of a molecule. This includes several enzyme families such as Cytochrome P450 Monooxygenases, Glutathione S-Transferases, Glucosyl transferases and several transporters. These enzymes are not specific to herbicide detoxification but serve other functions as well. This makes these groups of detoxification methods widely available among different weeds (Powles and Yu, 2010). While resistance factors are usually lower compared to TSR they are often very broad, making them potentially active to yet unknown MoA (Délye et al., 2011).

Resistance to ALS- and ACCase-Inhibitors

Resistance to ACCase-inhibitors by TSR in *A. myosuroides* is conferred by several different mutations at five different SNPs, namely I1781, W2027, I2041, D2078 and G2096 (Délye et al., 2005). Several cases of *A. myosuroides* were also reported elsewhere e.g. Menchari et al. (2006); Balgheim (2009); Délye et al. (2010b); Knight (2016). Menchari et al. (2007a) reports that resistance by a mutation at D2078 confers a fitness penalty for the plant, leading to a significant reduction in biomass, height and seed production. Despite resistance by TSR there many EMR cases are also reported (Petit et al., 2010; Délye et al., 2011; Cummins et al., 2013). Délye et al. (2010b) found that among several samples analyzed from all over Europe the majority did not have a target-site mutation as confirmed by Moss et al. (2007) for the UK. For samples from the UK Knight (2016) found in a non-random sampling from 2011 that target site mutations were found in almost all samples.

Resistance to ALS-inhibitors is already reported at several locations, while being shorter on the market compared to the ACCase-inhibitors (Marshall and Moss, 2008; Knight, 2016). As for ACCase-inhibitors both types of mechanisms TSR and EMR can occur sometimes even within the same plant (Knight, 2016). Marshall and Moss (2008) reports mutations at P197 and W574 as responsible for the loss of efficacy. Moss and Perryman (2007) already reported problems with the control of *A. myosuroides* using flupyrsulfuron at its market introduction, highlighting that EMR which was most likely selected with other MoA is also a big problem for this MoA.

Resistance to both ACCase- and ALS-inhibitors occurs in all key countries where *A. myosuroides* occurs. Moss and Perryman (2007) saw an increase in the resistance spread and the majorities of samples showing resistance to ACCase-inhibitors which led to the authors to conclude that due to the high use of ALS-inhibitors the problems to this MoA will arise in the near future. Knight (2016) found in his sampling that a high number of samples are already resistant to both MoA. No reports about a fitness penalty with either mechanism have been found for *A. myosuroides* and other grasses so far. The frequency and distribution of resistance will be discussed in Chapter 2 in relation to the data of this study.

1.2.4 Development of herbicide resistance

While research on the mechanism of herbicide resistance is only one part of resistance research, understanding the driving factors behind has recently gained more attention (Neve et al., 2009, 2014). Booth and Swanton (2002) describes the evolution of a (resistant) population as a passing through filters suggesting that if the same species is selected in different locations under similar environments it will posses the same (resistance) characteristics. The term location in this context refers to a geographical location with an environment and describes the habitat including its management by the farmer. Different populations might therefore evolve with the same resistance pattern based on redundant evolution (Délye et al., 2010b). Before relating the habitat to the resistance status, the suitability of a habitat for a given weed species needs to be identified. Habitat suitability, in allowing large population numbers (high abundances & densities) at a given mutation rate , is essential in the development of herbicide resistance (Powles and Yu, 2010; Jasieniuk et al., 1996). In other words habitats (including management by the farmer) that allow high weed infestation have an increased risk of herbicide resistance development compared to systems with lower infestations.

In organisms with limited mobility, distinctions between two populations can arise according to distance (isolation by distance) and/or environment (isolation by environment). Tiffin and Ross-Ibarra (2014) reports that isolation both by distance and by environment plays a role in the final structure of a population, with isolation by distance being of greater importance based on a recent meta analysis. The authors use this to conclude that an adaptive trait that occurs as a response to a certain environment can be used to 1) understand the adaptive capacity of a gene 2) help in the understanding of evolutionary constraints in evolution and 3) help to improve the prediction of such adaptive responses to selection. With regard to herbicide resistance several mutations responsible for target-site mutations have been identified already. With regards to EMR the identification of genes and their function is still ongoing e.g Gaines et al. (2014). Regional variation that results in differences in the environment might therefore be essential in the development of herbicide resistance and result in a "mosaic pattern" (Baucom and Mauricio, 2007). To fully understand the picture of herbicide resistance adaptation, Neve et al. (2014) calls for more empirical studies on a broad level considering both management aspects and the temporal and spatial dynamics of herbicide resistance. According to these authors, emphasis should be put again on the ultimate causes of resistance development rather than the outcome of the selection process. Farmers worldwide might benefit from such research as it enables agricultural decision makers to detect resistance development early and take counteractive measures at the early stages of the development process which are considered to be most crucial (Neve et al., 2009). While these are all general considerations, research to differentiate TSR and EMR has shown that low (sub-lethal) doses in particular are responsible for the enhancement of EMR (Neve and Powles, 2005a). However, Yu and Powles (2014) points out that sub-lethal doses result in the selection of both EMR and TSR.

1.3 Goals of the Study

The goals of the study were to assess the status of herbicide resistance to both ACCase and ALS-inhibitors at a defined area in Southern Germany where resistance has been confirmed in a few fields. The study attempted to gain insights into the temporal and spatial dynamics of herbicide resistance to both Modes of Action. Intensive interviews with farmers were carried out to analyze the causes of resistance and identify the main factors behind herbicide resistance development. Based on these findings, the prediction of herbicide resistance for the development of a herbicide resistance prediction tool was attempted.

1.4 Thesis Layout

The work is divided into three main chapters (Chapter 2, 3 and 4).

Chapter 2 analyzes the temporal and spatial variability of *A. myosuroides* resistance to ACCase-inhibitors and ALS-inhibitors . The relationship between years and fields is established and data linked to the fields location.

Chapter 3 analyzes field management information obtained from farmer interviews. The data is linked to the ALS-inhibitor resistance status. Furthermore, the soil characteristics are analyzed to identify soils with a higher *A. myosuroides* carrying capacity. The relationship between farm management, soil characteristics and resistance is analyzed.

Chapter 4 uses the key features identified in chapter 3 to predict the resistance situation as observed in chapter 2. The data should give further insights into the resistance development of *A. myosuroides* to ALS-inhibitors . Two approaches are used to achieve this: a deterministic simulation model and a supervised learning technique.

Chapter 2

Temporal and Spatial Evolution of Herbicide Resistance to ACCase- and ALS-Inhibitors at the Field Level in *Alopecurus myosuroides* Huds.

2.1 Introduction

Herbicide resistance of *A. myosuroides* to either ACCase-inhibitors and ALS-inhibitors or both is seen in several European countries (Heap, 2015). This causes a great problem for cereal farmers where those Modes of Action (MoA) are mostly applied for selective *A. myosuroides* control. Reports show that both TSR and EMR occur against either MoA (Délye et al., 2004; Menchari et al., 2006; Knight, 2016). In a comparison of multiple studies Moss et al. (2007) reported resistance to the ACCase-Inhibitor fenoxaprop-P in >80% of the samples collected in 2002 and 2004. In a sampling by Menchari et al. (2006) *A. myosuroides* was found in 80% of the sampled fields with 65% containing at least one mutation at the ACCase enzyme. Délye et al. (2010b) found in a comparison of different samples from non-random sampling in the UK, France and Germany that most plants have resistance to ACCase-inhibitors , attributed mostly to EMR. Since resistance to ACCase-inhibitors occurs widely for this 30+ year old MoA the question remains to which extent resistance to the newer ALS-inhibitors can be observed. Knight (2016) found that in 90% of the samples analyzed from across the UK resistance to an ALS-inhibitor was found. This is an alarming signal as resistance to this MoA will greatly reduce the available options for farmers to control *A. myosuroides* in the field. The sampling however focused on fields with a reported lack of ALS-inhibitors efficacy and was not based on random sampling, precluding true prevalence estimation.

To analyze the possible extent of resistance, proactive analysis of fields on a random basis is required to detect selection processes before an outbreak in the field. Various studies have analyzed the distribution of herbicide resistance in broader geographical areas with different focuses and different weedy species (Menchari et al., 2006; Baucom and Mauricio, 2007; Okada et al., 2013; Délye et al., 2015; Knight, 2016). These studies are important as they describe the current extent of herbicide resistance. Non-random sampling however prevents an accurate assessment of the genetic variability at a given region, as discussed by Menchari et al. (2006) and Knight (2016). Furthermore, the comparison with other areas is limited as the genetic variation at the given location cannot be properly assessed. None of these studies focuses on the development of resistance over time by continuously sampling a field with low or no resistance over at least 2 years or by re-sampling fields on a broader scale to validate their findings. While this may be a minor aspect in simplified systems such as RoundUp-Ready crops with mono-culture or orchard like systems, it is important for the diverse cropping systems found in Western Europe. In addition, the occurrence of multiple resistances in the same population are rarely studied together. Despite their limitations, all studies found a great variability in the occurrence

of resistance patterns at the scales analyzed. Most authors related this to the great diversity of selection pressures, as suggested by Jasieniuk et al. (2008). The attempt was to properly address the resistance status of a field over time with the focus on abundance of resistance, spread and temporal variability. The analysis of numerous fields was started in three distinct but proximate locations with each one confirmed resistance case. The local spread and variability of resistance was assessed and compared between locations to analyze whether isolation by distance occurs (Tiffin and Ross-Ibarra, 2014). The analysis focused on two MoA ACCase- and ALS-Inhibitors. The aim of this chapter is not to confirm of fields with reduced efficacy and their outcome, but to observe an ongoing selection pressure and characterize the underlying evolutionary factors as proposed by Neve et al. (2014). Open points which currently published studies cannot answer are thus the focus of this chapter. These are:

- **The level of *A. myosuroides* infestation at locations with confirmed resistance to one or two MoA**

  While weed populations over wide geographical areas have been sampled and compared, a complete sampling of all fields in a defined radius with focus on the development of a population (and resistance status) over multiple years has not been carried out. The assessment of the resistance status of multiple fields from the same farm and the comparison with neighboring farms has not yet been done. It is assumed that while various fields are exposed to the same environmental factors (e.g. soil) their management might vary according to farmer management. The advantage of having three locations in which multiple fields are sampled allows the comparison of findings at one location with those at the other. This separates local influences from geographic distance, directing resistance research towards comparisons of isolation by distance vs. isolation by environment (Tiffin and Ross-Ibarra, 2014). In an extensive sampling approach before the time of herbicide use Fruwirth (1908) observed geographical areas with higher and lower abundances of *A. myosuroides* without further specifying the causes for his observations. In a more detailed analysis on an individual field level Balgheim (2009) were able to show that both resistant and sensitive *A. myosuroides* occurs in patches within one field, suggesting heterogeneous conditions. This finding is important as it indicates that different plant numbers occur within fields, subsequently leading to differences in the number of resistance genes (EMR) or alleles (TSR) present at the beginning of herbicide selection. Proper identification of abundance levels is therefore important in observational studies especially on resistance development. Different crops and the

resulting herbicide regime, however, offer different opportunities for *A. myosuroides* to develop with winter sown crops showing generally higher infestation levels than summer crops (Menck, 1968). Based on these findings it is assumed that infestation level of *A. myosuroides* will be very heterogeneous as multiple factors such as crop rotation and other environmental factors come together.

- **The abundance of resistance at locations with confirmed resistance to one or two MoA**

  As every location studied includes a single case of confirmed resistance to either one or two MoA the absence of further farmer complaints would suggest a rather low overall resistance level. In studies by Menchari et al. (2006) and Knight (2016) the overall resistance level was found to be high for either ACCase-inhibitors or both ACCase-inhibitors and ALS-inhibitors . All studies were conducted at a larger scale and focused on fields with suspected loss of herbicidal efficacy. Diversity in crop rotations and the relationship of the *A. myosuroides* growth stage with the development status of the crop (Menck, 1968) might make the exchange of pollen flow between fields an exception. It is assumed that different stages of the selection process of both MoA in the development will be encountered. The selection to ACCase-inhibitors might be further evolved compared to the selection to ALS-inhibitors based on the findings presented earlier. Different levels of accumulation of resistance genes (EMR) and/or alleles (TSR) might serve as an indicator of the variety of the underlying factors involved in resistance selection.

- **The genetic diversity with regard to resistance in one location across all the fields studied**

  A. myosuroides is an out-crossing species. Studies by e.g. Délye et al. (2010a) imply that pollen flow is high and resistance spread occurs via pollen flow from a resistant source to a susceptible source; here the question of the actual relevance of this phenomenon shall be explored further. Sampling of multiple fields within a small area permits the analysis of the genetic diversity at the field and/or farm level. Diversity in resistance patterns is related to the diversity of resistance mechanisms observed Jasieniuk et al. (2008) . Given this conclusion, the diversity in a heterogeneous management landscape within a comparable environment would be high between farms but low within a farm. The relation of findings for each farmer in comparison to neighbors might thus be of particular interest. Both qualitative and quantitative occurrence of factors might therefore be assessed. This gives greater

insights and allows the study of the impact of a simplified ecosystem (agriculture) on the genetic diversity of resistance alleles.

2.2 Material & Methods

2.2.1 Site Selection

Three initial fields in the area of Hohenlohe (NE Baden-Württemberg, Germany) were reported by farmers with herbicide failure in 2009. Resistance to ALS-inhibitors was found in two of them with all three showing resistance to ACCase-inhibitors . These three fields served as a starting point and conferred the denomination of the three locations H, M and Z, respectively. The choice was based on the different patterns towards ALS-inhibitors observed. The sample from location H showed no resistance to ALS-inhibitors , the sample from location M contained a mutation at position W574X and the sample from location Z contained a mutation at position P197X. The three fields are approximately 10-20km apart from each other (figure 2.1).

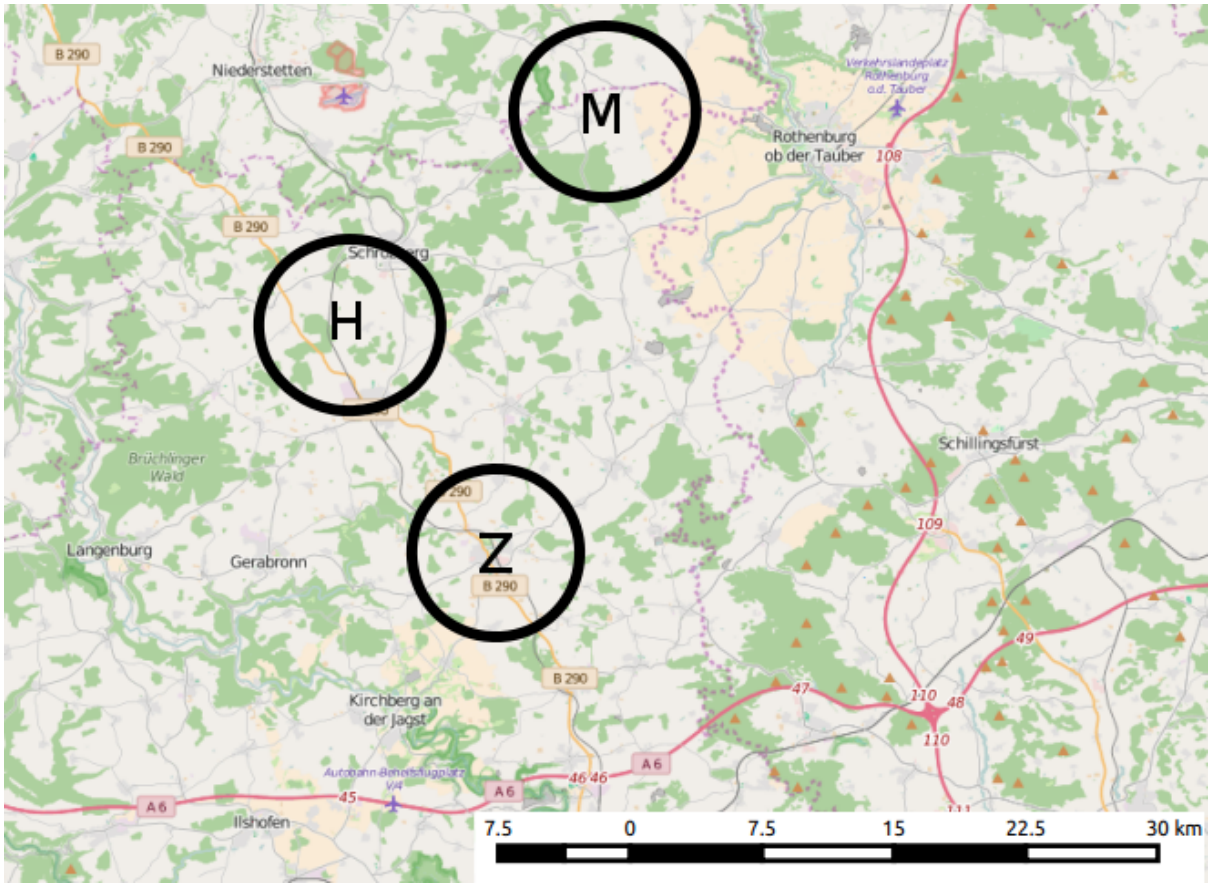


Figure 2.1: Excerpt of the Hohenlohe area with the three locations H, M and Z in circles

Sampling was conducted between 2010 and 2015 with the number of chosen fields being extended in the years 2011 and 2012 at each of the three sampling locations. Selection of fields, except the three initial fields, was based on common borders with the three

starting points. Farmers who owned those and their other fields set the dimensions for the sampling area. Attempts were made to sample several fields for each farmer but all fields within geographical zones lay within the borders made by main roads, forests or rivers. Currently, observations from 1232 fields are included in the study. The number of observations per field and those fields that were sampled consecutively (without missing values in between) are shown in table 2.1. The number of fields and the repeated sampling on several of them makes a total of 2249 site-years and an area of 6111ha being sampled.

	No. of Observations	1	2	3	4	5	6	Sum
1	Consecutive		179	69	51	24	22	345
2	Total	701	260	122	70	50	22	1225

Table 2.1: Number of fields sampled. Column names indicate the number of observations with the corresponding number of fields. Consecutive indicates the number of fields that were continuously sampled without missing years in between.

General Overview of Harvested Fields

The median field size at the three locations H, M and Z was 4.3ha, 4.1ha, 3.0ha respectively, indicating many fields in a divided landscape. However, there were a few fields that were larger than 20ha but those were very often divided into smaller fields over the study period or over some years. If fields were divided within one or several years they were regarded as separate samples. Between five and 10 fields or 20-50ha per farmer were typically sampled. Multiple fields per farmer enabled the differentiation of each farmers management from environmental factors. There was also a larger number of farmers where no more than two fields were sampled. These farmers farm mostly outside the main sampling area. Another reason is the partly present part time farming for some farmers who only have a small number of fields.

2.2.2 Seed Sampling

Seed sampling was done during the months of June, July and August when most *A. myosuroides* plants set ripe seeds and the crops are not yet harvested. All herbicide applications by farmers had been carried out prior to the time of sampling. Samples were taken by walking two to four tractor tracks and sampling two borders-sides. Where possible, all seeds from at least 100 ears of different plants along the tractor tracks were taken. Deviations from that routine were only applied for oilseed rape and other pulses with dense crop stands that could not be entered. Samples were then taken

from individual patches with poor crop stands or field borders. Unsprayed patches were sampled and assessed separately but are not considered in this study. The *A. myosuroides* infestation of the fields was recorded using a six-level rating scale adopted from Chancellor and Froud-Williams (1984) (table 2.2). In addition records of abundance for other weeds were taken but are not reported here. Infestation levels of 4 and 5 are considered to cause yield loss. Among fields with infestation level 0, incorrect classification may occur as plants can been overlooked due to the low infestation levels. It is therefore possible that a more intense sampling of these fields would have produced more cases with an infestation level of 1. There was no deviation from the sampling routine for these samples.

Infestation levels	Description
0	No plants found
1	Few individual plants found
2	Smaller patches/spots found
3	Larger patches found. Infestation up to 50% of the field
4	Infestation of the entire field with up to 50 ears*m^{-2}
5	Infestation of the entire field with up to 100 ears*m^{-2}

Table 2.2: Infestation levels used to assess *A. myosuroides* infestation in the field. Infestation levels of 4 & 5 are considered to cause yield

Table 2.3 shows the distribution of samples across crops in the different years. The top five crops are winter wheat, corn, winter and summer barley and oilseed rape. However, as with summer barley and other summer crops (excluding maize) their proportion is substantially higher in 2012 where a strong winter with low freezing temperatures and no snow coverage caused failure of the winter crop and led to subsequent seeding of a summer crop. A strong increase of corn is observed starting in 2012 and continuing until 2014.

Leguminous crops such as *Vicia fabae*, *Pivum sativum* and *Glycine max* are rarely observed as the majority of farms rear animals and therefore prefer to grow crops with higher fertilizer input. Furthermore, specialty crops such as sugar beet and potatoes are not very common and only grown by some farmers. 11 fields were excluded from the analysis as they were continuously kept as grassland or fallow and no *A. myosuroides* was found on them.

2.2.3 Greenhouse Bioassay

Seeds were stored in the cold-room after harvest for at least four weeks (4 °C). Afterwards they were grown in 8 cm pots (Fa. Jiffy) filled with standard field soil (loamy silt) using two repetitions each. About 30 plants per pot were grown. The pots were placed

Crop	2010	2011	2012	2013	2014	2015	Total
AVESA	7	5	28	6	6	6	58
	7.5%	5.9%	6.8%	1.4%	1.2%	0.8%	
BRSNW	2	18	31	34	38	43	166
	2.2%	21.2%	7.5%	8.1%	7.3%	6.0%	
HORVS	11	8	168	13	14	8	222
	11.8%	9.4%	40.6%	3.1%	2.7%	1.1%	
HORVW	18	22	2	44	89	136	311
	19.4%	25.9%	0.5%	10.5%	17.1%	18.9%	
Others	1	1	18	23	26	34	103
	1.1%	1.2%	4.3%	5.5%	5.0%	4.7%	
Pulses	0	0	4	4	9	10	27
	0.0%	0.0%	1.0%	1.0%	1.7%	1.4%	
TRZAS	0	0	81	8	4	0	93
	0.0%	0.0%	19.6%	1.9%	0.8%	0.0%	
TRZAW	49	25	32	160	177	151	594
	52.7%	29.4%	7.7%	38.3%	34.1%	21.0%	
TTLWI	1	1	0	3	10	37	52
	1.1%	1.2%	0.0%	0.7%	1.9%	5.1%	
ZEAMX	4	5	50	123	146	295	623
	4.3%	5.9%	12.1%	29.4%	28.1%	41.0%	
Total	93	85	414	418	519	720	2249
	4.1%	3.8%	18.4%	18.6%	23.1%	32.0%	

Table 2.3: Distribution of samples across crops in different years. First row indicates the number of crops sampled. Second row indicates the frequency of the observation in the given year.

in a glasshouse with 60% humidity and a temperature of 22℃ at day time and 15℃ at night time. 12h light with a minimum of $2200\mu E/m^2s$ at 555nm were provided (high pressure lamps were turned on if necessary). A 8002 nozzle was used for applications with $300l/ha$ of water at 2hPa. Plants were sprayed together with a sensitive greenhouse standard (Appels Wilde Samen GmbH, Darmstadt, Germany) at BBCH 11/12. Different rates of commercial herbicides were assessed as shown in table 2.4. An unsprayed control treatment was kept for every biotype. Plants were rated three weeks after application with survivors and dead plants counted. A survivor is denoted as a plant showing regrowth after herbicide application at the time of rating. The control of the herbicide was calculated based on those values. Survivors were kept for further laboratory analysis. Trade names rather than active ingredients are given in the text since some products contain multiple active ingredients

Product	Active Ingredient	Formulation	Use Rate
Atlantis	Mesosulfuron/Iodosulfuron+Mefenpyr	WG	250 g/ha
Atlantis	Mesosulfuron/Iodosulfuron+Mefenpyr	WG	500 g/ha
Atlantis	Mesosulfuron/Iodosulfuron+Mefenpyr	WG	1000 g/ha
Focus Ultra	Cycloxydim	EC	2.5 l/ha
Ralon Super	Fenoxaprop	EC	2.4 l/ha
Axial	Pinoxaden	EC	1.2 l/ha
MaisTer fluessig	Foramsulfuron/Iodosulfuron/Isoxadifen-ethyl	OD	1.5 l/ha

Table 2.4: Treatment List of products with their respective active ingredient, formulation and use rate used in greenhouse bioassays

2.2.4 Laboratory Analysis

Collected leaf material from step 2.2.3 was further analyzed in the lab by using pyrosequencing to detect target site mutations as described in Beffa et al. (2012) and Riggins et al. (2010). The ALS enzyme was sequenced for mutations at positions P197 and W547. The ACCase enzyme was sequenced for mutations at position I1781, W2027, I2041, D2078 and G2096. Mutation at these sites where previously reported to confer resistance in *A. myosuroides* (Délye et al., 2004; Heap, 2015). Plants surviving the greenhouse treatment but not carrying a target site mutation were considered to have EMR.

2.2.5 Statistical Analysis

All Statistical analyses were carried out using R 3.2.3 (R Core Team, 2016). Field infestation levels were standardized by mean and standard deviation per crop and year to

allow the comparison of infestation levels between neighboring fields with differently sown crops. If not otherwise stated, the reference to wheat, barley and oilseed rape is to the winter sown form. Greenhouse data was grouped into "high" (>80% control), "reduced" (50-80% control) and "low" (<50% control). Abundances were compared across locations and products. A comparison of abundances was done using Chi-Square test with simulated p-values using 9999 permutations. Correlations across products were tested using Pearson's correlation coefficient with a significance level of $\alpha 0.05$. An ANOVA on the influence of crops and infestation values was carried out on absolute control values by the herbicide. Abundances of target site mutations were also assessed using the Chi-Square test with simulated p-values using 9999 permutations as described earlier.

Analysis of Populations

The relation between populations was assessed in different ways. The first approach focussed on the ACCase locus. Pairwise Fst values between two samples to assess genetic variation at this enzyme were calculated using the pp.fst function from package hierfstat for mutations related to the ACCase enzyme (Weir and Cockerham, 1984; Goudet and Jombart, 2015). Values were then assessed for spatial auto-correlation using the Mantel Test (Mantel, 1967) with 9999 permutations as implemented in the ade4 package via the mantelr.test function Dray (2007).

In a second approach the full set of data was assessed via cluster analysis to estimate the potential similarity of the sampled fields. The assessed data comprised 1) the standardized infestation values, 2) the control rates of 500g/ha Atlantis WG and 2.4l/ha Ralon Super in the greenhouse and 3) the frequency of alleles at all mutations assessed via pyrosequencing for each year of a field in which this entire set of data was available. Only complete observations were considered; observations where not enough seeds could be harvested in the field to run all tests were discarded. Fields with only one observation were not considered in the analysis. The dataset was assessed using manova (Pillai) with significant factors being used to standardize the individual observations by mean and standard deviation. The mean and standard deviation were calculated across all observations of a given field to reduce multiple observations into one (dimension reduction). Clustering was performed on the observations using Manhattan distance and the ward.D2 algorithm both implemented in R. Hierarchical Clustering via multiscale bootstrap resampling was applied by using the pvclust function of the pvclust package with 9999 permutations (Suzuki and Shimodaira, 2015). Clusters with an approximately unbiased p-value of 0.8, 0.9 and 0.95 were extracted and fields within the clusters recorded.

A similar approach was followed to assess the variability within the observations over time. Standardized values for each observation were used for hierarchical clustering via multiscale bootstrap resampling for the observations, without the dimension reduction described above. Fields occurring at least twice within the same cluster with an approximately unbiased p-value of 0.8 were recorded. These records were related to the mean infestation level of *A. myosuroides* in the field and the remaining efficacy of *A. myosuroides* in the greenhouse.

2.3 Results

2.3.1 Infestation

Infestation after herbicide application by the farmer was low in the majority of fields (table 2.5). Almost all farmers spray against *A. myosuroides* , and so in the majority of cases the herbicide application was still satisfactory. No field in agricultural use showed an infestation level of 0 in all sample years, indicating that *A. myosuroides* was present on each field. No records of the infestation prior to herbicide application exist. About 75% of the observations were within the infestation level 0 or 1, with only 7% within infestation levels 4 or 5 and of agronomic relevance. The frequency of observations of infestation level 2 and higher is relatively constant over the years. Some variation is observed between the frequencies of infestation level 0 and 1. However, the frequencies of both infestation levels combined are relatively stable.

Significant differences between sampling years were found for the infestation levels observed (Chi-Square: $\tilde{\chi}^2_{(20,\ N=1540)}$=99.68, p<.001) which is very common for agriculture. The comparisons of all pairs of years assessed using Fishers Exact test showed significant differences at α=.05 for all combinations except those between 2011-2014 and 2013-2015.

A significant effect between sampling locations was also observed (Chi-Square: $\tilde{\chi}^2_{(15,\ N=2249)}$ =85.8, p<.001, table 2.5). The differences were statistically significant for all comparisons (Fisher Exact Test p<0.01). While 23.3% of the samples in location H contain a higher infestation level than 1, 38.4% and 32.3% of samples had an infestation level higher than 1 in location M and Z respectively. The frequencies of fields with infestation levels of 4 and 5 is lower in location H than in M and Z (4.6% compared to 9.6% and 7.1% respectively)

Significant differences were also observed across the main crops (Chi-Square: $\tilde{\chi}^2_{(25,\ N=2249)}$ =174.3, p<.001). Only the comparisons between BRSNW vs. HORVW and HORVW vs. TRZAW were not significantly different from each other. ZEAMX for example showed relatively low infestation levels with fewer fields having high infestation levels. Only 17% of the observations for this crop exceeded the infestation level 1. This was different to BRNSW, HORVW and TRZAW where 24.7%, 42.5%, 36.4% exceeded that level.

Comparing infestation levels between neighboring fields with differently sown crops is not a straight forward approach. Differences in abundance after herbicide application by the farmer might be attributed to the crop and the underlying herbicide regime. Therefore an infestation level of 3 or more for corn, where *A. myosuroides* usually does not occur, might reveal more about the severity of the *A. myosuroides* abundance in the field

Year	0	1	2	3	4	5	Total
2010	41	31	10	7	3	1	93
	44.1%	33.3%	10.8%	7.5%	3.2%	1.1%	4.1%
2011	8	48	14	4	7	4	85
	9.4%	56.5%	16.5%	4.7%	8.2%	4.7%	3.8%
2012	112	198	61	17	22	4	414
	27.1%	47.8%	14.7%	4.1%	5.3%	1.0%	18.4%
2013	78	202	56	49	25	8	418
	18.7%	48.3%	13.4%	11.7%	6.0%	1.9%	18.6%
2014	60	294	92	40	27	6	519
	11.6%	56.6%	17.7%	7.7%	5.2%	1.2%	23.1%
2015	117	381	95	83	36	8	720
	16.2%	52.9%	13.2%	11.5%	5.0%	1.1%	32.0%
Total	416	1154	328	200	120	31	2249

Location	0	1	2	3	4	5	Total
H	230	433	100	61	32	8	864
	26.6%	50.1%	11.6%	7.1%	3.7%	0.9%	38.4%
M	51	264	86	62	39	10	512
	10.0%	51.6%	16.8%	12.1%	7.6%	2.0%	22.8%
Z	135	457	142	77	49	13	873
	15.5%	52.3%	16.3%	8.8%	5.6%	1.5%	38.8%
Total	416	1154	328	200	120	31	2249

Crop	0	1	2	3	4	5	Total
BRSNW	18	107	24	7	8	2	166
	10.8%	64.5%	14.5%	4.2%	4.8%	1.2%	7.4%
HORVW	24	155	60	40	22	10	311
	7.7%	49.8%	19.3%	12.9%	7.1%	3.2%	13.8%
Other	106	265	100	41	36	7	555
	19.1%	47.7%	18.0%	7.4%	6.5%	1.3%	24.7%
TRZAW	81	297	84	81	39	12	594
	13.6%	50.0%	14.1%	13.6%	6.6%	2.0%	26.4%
ZEAMX	187	330	60	31	15	0	623
	30.0%	53.0%	9.6%	5.0%	2.4%	0.0%	27.7%
Total	416	1154	328	200	120	31	2249

Table 2.5: Distribution of Infestation levels after all treatments by the farmer were carried out. Infestation Levels are ordered by Year (top), Location (middle) and Crop (bottom). The first row indicates the cases of individual infestation levels. The second indicates the frequency of observations.

than an infestation level of 3 or more in winter barley or winter wheat. To account for that, every observation was standardized by mean and standard deviation across all fields with the same crop from that sampling year representing a relative infestation (scaled Infestation). Mean values per field were calculated from these standardized infestation levels indicating the average relative infestation. The 10%, 25%, 75% and 90% quantile of the mean standardized infestation levels were calculated and fields represented accordingly (figure 2.2). The figure, representing the core fields of the three locations, indicate the relatively lower infestation in location H compared to location M and Z. The very high infestation level in location M is also well represented by the relative infestation across years. Furthermore all regions show individual or patches of fields with high relative infestation levels next to fields with low relative infestation. In all three regions, there seems to be no pattern in the occurrence of *A. myosuroides* based on the relative abundance of the weed at harvest.

2.3.2 Greenhouse Results

To assess the remaining herbicide efficacy of field survivors, where seeds that replenish the soil seed bank were produced, commercially available herbicides were used. Table 2.6 indicates the distribution across rating categories for the two ALS-inhibitors tested. Overall 39.3%, 32.0% and 27.6% of the samples were found to show low efficacy to Atlantis WG at 250g/ha, 500g/ha and 1000g/ha respectively while 19.9% showed low efficacy to MaisTer fluessig . MaisTer fluessig is thus more active across more samples than Atlantis WG across the 1240 samples analyzed. Meanwhile, increased dose rates of Atlantis WG gave better efficacy, indicated by the number of samples being rated with high control. Testing the abundance of the three rating groups across locations revealed that most comparisons of pairs are significantly different from each other (table 2.7). No significant difference was found between location H and location Z Atlantis WG at 250g/ha and no difference was found between location H and Z for 1000g/ha of Atlantis WG and 1.5l/ha of MaisTer fluessig . The remaining efficacy was found to be significantly lower for MaisTer fluessig at these two locations than at location M. The lowest remaining efficacy for all products was found at location M. For location M the difference between 500g/ha Atlantis WG , 1000g/ha Atlantis WG and 1.5l/ha MaisTer fluessig was not found to be significant while the comparison was significant at the other two locations. No significant difference was found between 500g/ha Atlantis WG and 1000g/ha Atlantis WG at location Z.

The comparison of the tested ACCase-inhibitors revealed that 93.0% and 83.3% of the samples showed low efficacy to Ralon Super and Axial respectively (table 2.8). The

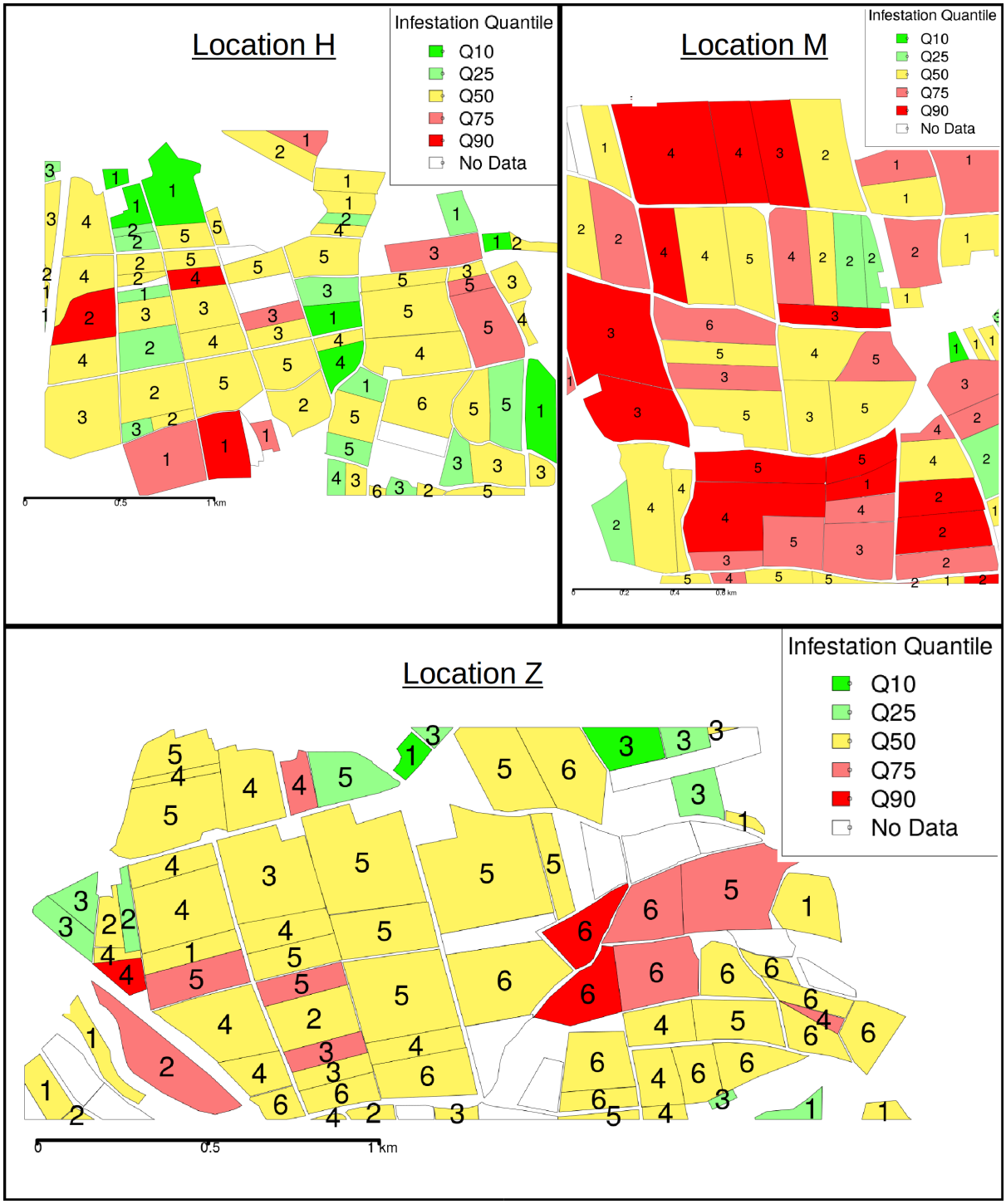


Figure 2.2: Maps of relative infestation levels across all samples for fields in the core area of location H, M and Z. Q10= <10% quantile, Q25= <25% quantile, Q50 = 25% <x >75% quantile, Q75= >75% quantile, Q90= >90% quantile. The number of observations considered is indicated for every field.

Herbicide	Rating	H	M	Z	Total
Atlantis WG 250g/ha	High	157	84	271	512
		30.7%	16.4%	52.9%	41.3%
		42.2%	23.7%	52.8%	
	Reduced	88	61	92	241
		36.5%	25.3%	38.2%	19.4%
		23.7%	17.2%	17.9%	
	Low	127	210	150	487
		26.1%	43.1%	30.8%	39.3%
		34.1%	59.2%	29.2%	
Atlantis WG 500g/ha	High	213	99	333	645
		33.0%	15.3%	51.6%	52.0%
		57.3%	27.9%	64.9%	
	Reduced	67	65	66	198
		33.8%	32.8%	33.3%	16.0%
		18.0%	18.3%	12.9%	
	Low	92	191	114	397
		23.2%	48.1%	28.7%	32.0%
		24.7%	53.8%	22.2%	
Atlantis WG 1000g/ha	High	245	119	358	722
		33.9%	16.5%	49.6%	58.2%
		65.9%	33.5%	69.8%	
	Reduced	49	56	71	176
		27.8%	31.8%	40.3%	14.2%
		13.2%	15.8%	13.8%	
	Low	78	180	84	342
		22.8%	52.6%	24.6%	27.6%
		21.0%	50.7%	16.4%	
MaisTer fluessig 1.5l/ha	High	303	129	437	869
		34.9%	14.8%	50.3%	70.1%
		81.5%	36.3%	85.2%	
	Reduced	31	56	37	124
		25.0%	45.2%	29.8%	10.0%
		8.3%	15.8%	7.2%	
	Low	38	170	39	247
		15.4%	68.8%	15.8%	19.9%
		10.2%	47.9%	7.6%	
	Total	372	355	513	1240
		30.0%	28.6%	41.4%	

Table 2.6: Greenhouse efficacy ratings for the two ALS-inhibitors tested with seeds from field survivors based in 1240 samples. The first row indicates the absolute number, the second indicates row frequencies and the third row indicates column frequencies for location H, M and Z separately.

Product		Atlantis WG 250g/ha			Atlantis WG 500g/ha			Atlantis WG 1000g/ha			MaisTer fluessig 1.5l/ha		
		H	M	Z	H	M	Z	H	M	Z	H	M	Z
Atlantis WG 250g/ha	H	-	*	.01	*	*	*	*	*	*	*	*	*
	M	*	-	*	*	**.33**	*	*	.01	*	*	*	*
	Z	.01	*	-	**.32**	*	*	*	*	*	*	*	*
Atlantis WG 500g/ha	H	*	*	**.31**	-	*	.04	.05	*	*	*	*	*
	M	*	**.33**	*	*	-	*	*	**.25**	*	*	.05	*
	Z	*	*	*	.04	*	-	**.9**	*	**.06**	*	*	*
Atlantis WG 1000g/ha	H	*	*	*	.04	*	**.9**	-	*	**.22**	*	*	*
	M	*	.01	*	*	**.26**	*	*	-	*	*	**.71**	*
	Z	*	*	*	*	*	**.06**	**.22**	*	-	*	*	*
MaisTer fluessig 1.5l/ha	H	*	*	*	*	*	*	*	*	*	-	*	**.31**
	M	*	*	*	*	.05	*	*	**.71**	*	*	-	*
	Z	*	*	*	*	*	*	*	*	*	**.31**	*	-

Table 2.7: P-Values for all pairwise comparisons from table 2.6. Reported p-Values were analyzed using a Chi-Square Test. P-Values were calculated using Monte Carlo Simulation with 10000 runs. *= p <.001

remaining efficacy of Focus Ultra was higher with only 16% of the samples showing low efficacy. The abundances of the three rating categories were not statistically different for Ralon Super across locations (table 2.9). For Axial the observations were significantly different for the comparison between location M and H but not for the other combinations tested. In addition, the comparison of location Z to location M was found not to be significantly different for Focus Ultra . A distribution of greenhouse efficacy for both Ralon Super and Atlantis WG across years and location for individual fields is shown in the maps of appendix 7.2.

Table 2.10 indicates that most correlations of the products tested were significant. The exception was the correlations between Focus Ultra and MaisTer fluessig and Focus Ultra to 500g/ha and 1000g/ha of Atlantis WG . The correlation between pairs of ALS-inhibitors and ACCase-inhibitors was always week (<0.17***) indicating a marginal relationship between these two MoA and the resistance patterns found in the study location. It indicates significant resistance to Ralon Super and Axial present in almost all samples, with resistance to ALS-inhibitors less frequently observed. The data also shows the tight linkage between the three dose rates of Atlantis WG . Compared to that the correlation of MaisTer fluessig to all of the Atlantis WG doses was lower. This, combined with data from table 2.6 indicates that more than one resistance pattern to ALS-inhibitors is present in our study location. A lower correlation of Focus Ultra to both Ralon Super and Axial in combination with data from table 2.8 indicates at least

Herbicide	Rating	H	M	Z	Total
Ralon Super 2.4l/ha	High	9	5	7	21
		42.9%	23.8%	33.3%	1.7%
		2.4%	1.4%	1.4%	
	Reduced	28	14	24	66
		42.4%	21.2%	36.4%	5.3%
		7.5%	3.9%	4.7%	
	Low	335	336	482	1153
		29.1%	29.1%	41.8%	93.0%
		90.1%	94.6%	94.0%	
Axial 1.2l/ha	High	34	12	27	73
		46.6%	16.4%	37.0%	5.9%
		9.1%	3.4%	5.3%	
	Reduced	39	34	61	134
		29.1%	25.4%	45.5%	10.8%
		10.5%	9.6%	11.9%	
	Low	299	309	425	1033
		28.9%	29.9%	41.1%	83.3%
		80.4%	87.0%	82.8%	
Focus Ultra 2.5l/ha	High	240	188	288	716
		33.5%	26.3%	40.2%	57.7%
		64.5%	53.0%	56.1%	
	Reduced	75	108	132	315
		23.8%	34.3%	41.9%	25.4%
		20.2%	30.4%	25.7%	
	Low	57	59	93	209
		27.3%	28.2%	44.5%	16.9%
		15.3%	16.6%	18.1%	
	Total	372	355	513	1240
		30.0%	28.6%	41.4%	

Table 2.8: Greenhouse efficacy ratings for the three ACCase-inhibitors tested with seeds from field survivors based on 1240 samples. The first row indicates the absolute number, the second row indicates row frequencies and the third indicates column frequencies for location H, M and Z separately.

Product		Ralon Super 2.4l/ha			Axial 1.2l/ha			Focus Ultra 2.5l/ha		
		H	M	Z	H	M	Z	H	M	Z
Ralon Super 2.4l/ha	H	-	**.07**	**.1**	*	**.45**	.01	*	*	*
	M	**.07**	-	**.91**	*	*	*	*	*	*
	Z	**.1**	**.91**	-	*	*	*	*	*	*
Axial 1.2l/ha	H	*	*	*	-	*	**.07**	*	*	*
	M	**.45**	*	*	*	-	**.21**	*	*	*
	Z	.01	*	*	**.07**	**.2**	-	*	*	*
Focus Ultra 2.5l/ha	H	*	*	*	*	*	*	-	*	.04
	M	*	*	*	*	*	*	*	-	**.31**
	Z	*	*	*	*	*	*	.04	**.32**	-

Table 2.9: P-Values for all pairwise comparisons from table 2.8. Reported p-Values were analyzed by using Chi-Square Test. P-Values were calculated using Monte Carlo Simulation with 10000 runs. *=p <.001

two resistance patterns against ACCase-inhibitors .

	Atlantis WG 250g/ha	Atlantis WG 500g/ha	Atlantis WG 1000g/ha	Axial 1.2l/ha	Ralon Super 2.4l/ha	Focus Ultra 2.5l/ha
Atlantis WG 250g/ha						
Atlantis WG 500g/ha	0.89***					
Atlantis WG 1000g/ha	0.84***	0.90***				
Axial 1.2l/ha	0.21***	0.15***	0.13***			
Ralon 2.4l/ha	0.17***	0.12***	0.10***	0.68***		
Focus2.5l/ha	0.08**	0.03	0.02	0.41***	0.34***	
Maister fluessig 1.5l/ha	0.70***	0.77***	0.82***	0.06*	0.06*	-0.02

Table 2.10: Correlation coefficient (r) of control data for two ALS-inhibitor and 3 ACCase-inhibitor tested in the greenhouse from seeds of field survivors. Significance is indicated with p<.001***, p<.01**, p<.05*

Figure 2.3 indicates that the crop sampled has an influence on the remaining efficacy found in the greenhouse. Typically the sampled crop had always a significant effect on the control observed in the greenhouse (table 2.11). No differentiation between infestation levels was found for Ralon Super , indicating that no matter how heavily infested the field was and which crop was sampled, the efficacy remained low.

For all other products tested, the infestation level was decisive, with lower infestation levels showing higher remaining efficacy. The remaining efficacy of Axial was comparable to Ralon Super at low levels. Somewhat better efficacy was typically observed in samples

where ACCase-inhibitors may not have been sprayed (TRZAW, ZEAMX). Focus Ultra showed the lowest remaining efficacy in samples from BRSNW compared to all other crops, with infestation levels only marginally significant (p=.034). Crops that are potentially sprayed with ALS-inhibitors (TRZAW, ZEAMX) show lower levels of greenhouse control to this MoA indicating that a pre-selection by the farmer left survivors with reduced efficacy. In comparison this is even more pronounced in ZEAMX than in TRZAW and other crops.

Product	Crop	Infestation	Crop*Infestation
Atlantis WG 250g/ha	**<.001**	**<.001**	**0.002**
Atlantis WG 500g/ha	**<.001**	**<.001**	**<.001**
Atlantis WG 1000g/ha	**<.001**	**<.001**	**<.001**
MaisTer fluessig 1.5l/ha 1.5	**<.001**	**<.001**	**<.001**
Ralon Super 2.4l/ha 2.4	**<.001**	.069	.336
Axial 1.2l/ha	**<.001**	**<.005**	.493
Focus Ultra 2.5l/ha	**<.001**	**.034**	.341

Table 2.11: ANOVA table with p-values of significant factors and their interaction from figure 2.3

Table 2.12 illustrates the previously mentioned pre-selection in the field on a few examples. For Field "H015" the infestation always remained low (except in 2011), with high efficacy for the ALS-inhibitors tested across crops in all years. In 2013, when BRSNW was grown, the efficacy of Focus Ultra drops sharply, indicating a shift of the survivors towards reduced efficacy of Focus Ultra . Similarly, in fields "Z014" and "Z023" a strong selection towards ALS-inhibitors in 2014 and 2013 was found respectively. This indicates strong selection by the farmer in the field as a result of a highly effective herbicide application, leaving only survivors that cannot be controlled by this MoA in the greenhouse. Problematic here is that the farmer will typically not notice the progressed selection in the field as the infestation level at harvest is rather low. In addition, the reduced efficacy was only found in one year while samples from other years do not show field survivors with low efficacy. It can be seen that while survivors found in "Z014" show resistance to all ALS-inhibitors , the efficacy of MaisTer fluessig in "Z023" is still high.

2.3.3 Laboratory Results

Samples of greenhouse survivors were analyzed in the laboratory for target site mutations conferring resistance to either ALS-inhibitors or ACCase-inhibitors . The aim of this subsection is to show a) the varieties of target site mutation found regardless of where they originated and b) to show the different selection pressures applied by the herbicides tested

Figure 2.3: Observed control to ALS-inhibitors and three ACCase-inhibitors tested in the greenhouse. Differences are shown across different crops and infestation levels. Red, yellow and green background color indicate the three rating groups, "Low", "Reduced", "High", that were used previously.

Field	Year	Crop	Infestation	Atlantis WG 250g/ha	Atlantis WG 500g/ha	Atlantis WG 1000g/ha	MaisTer fluessig 1.5l/ha	Ralon Super 2.4l/ha	Axial 1.2l/ha	Focus Ultra 2.5l/ha
H015	2011	AVESA	4	100	100	100	100	7	30	100
	2012	TRZAS	0							
	2013	BRSNW	1	94	100	100	100	6	8	56
	2014	TRZAW	1	81	99	99	100	0	17	98
Z014	2011	HORVW	2	81	82	79	100	3	0	32
	2012	TRZAS	1	100	100	100	100	0	10	71
	2013	TRZAW	2	0	14	0	18	18	50	50
Z023	2011	BRSNW	2	97	98	100	100	3	0	0
	2012	HORVS	1	91	94	96	100	4	0	30
	2013	TRZAW	1	26	25	44	100	29	7	0
	2014	ZEAMX	1	100	80	100	100	22	17	67

Table 2.12: Field Infestation level and control in the greenhouse [%] for three selected fields across multiple years.

in the greenhouse. This is important as it shows how a given herbicide regime applied by the farmer can influence the selection of target-site mutations. The frequencies presented reflect not the frequencies of a mutation in the field sample, but the frequencies among the survivors of a sample by a given greenhouse treatment. Seven SNPs were analyzed and several mutations found (table 2.13). These data indicate a great variability of target site mutations among the samples. Among the SNPs conferring resistance to ALS-inhibitors P197S, P197A, P197T and W574L were found. Among the SNPs conferring resistance to ACCase-inhibitors I1781L (by a substitution of A to C and A to T at the first position of the codon), I1781V, I1781T, W2027C (by a substitution of G to C and G to T at the third position of the codon), I2041V, I2041N, D2078G, G2096A were found. Among all positions analyzed, the sensitive genotype dominates, followed by one mutation that shows higher presence compared to the others where there are multiple. The higher variability of genotypes at position 197, 1781 and 2041 as indicated by the greater number of different substitutions, may result from the presence of exchanges in two nucleotide positions at these loci, compared to only one in all others. At position 2027 there are also two substitutions, though they both code for Cystein. Except W574 where 65% of the mutations were heterozygous, all other mutations showed a degree of heterozygocity >85%.

On an individual plant level we found that 33% of the plants surviving any of the

	Amino Acid Substitution							
Amino Acid	197	574	1781	2027	2041	2078	2096	Total
Ala	89	0	0	0	0	0	1099	1188
	0.4%	0.0%	0.0%	0.0%	0.0%	0.0%	5.1%	
Asn	0	0	0	0	1584	0	0	1584
	0.0%	0.0%	0.0%	0.0%	7.4%	0.0%	0.0%	
Asp	0	0	0	0	0	**18936**	0	18936
	0.0%	0.0%	0.0%	0.0%	0.0%	88.5%	0.0%	
Cys(C)	0	0	0	2739	0	0	0	2739
	0.0%	0.0%	0.0%	12.8%	0.0%	0.0%	0.0%	
Cys(T)	0	0	0	304	0	0	0	304
	0.0%	0.0%	0.0%	1.4%	0.0%	0.0%	0.0%	
Gly	0	0	0	0	0	2458	**20291**	22749
	0.0%	0.0%	0.0%	0.0%	0.0%	11.5%	94.9%	
Ile	0	0	**16831**	0	**19762**	0	0	36593
	0.0%	0.0%	78.9%	0.0%	92.6%	0.0%	0.0%	
Leu	0	5254	0	0	0	0	0	5254
	0.0%	24.5%	0.0%	0.0%	0.0%	0.0%	0.0%	
Leu(C)	0	0	145	0	0	0	0	145
	0.0%	0.0%	0.7%	0.0%	0.0%	0.0%	0.0%	
Leu(T)	0	0	4078	0	0	0	0	4078
	0.0%	0.0%	19.1%	0.0%	0.0%	0.0%	0.0%	
Pro	**19290**	0	0	0	0	0	0	19290
	90.2%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	
Ser	41	0	0	0	0	0	0	41
	0.2%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	
Thr	1972	0	214	0	0	0	0	2186
	9.2%	0.0%	1.0%	0.0%	0.0%	0.0%	0.0%	
Trp	0	**16149**	0	**18354**	0	0	0	34503
	0.0%	75.5%	0.0%	85.8%	0.0%	0.0%	0.0%	
Val	0	0	55	0	5	0	0	60
	0.0%	0.0%	0.3%	0.0%	0.0%	0.0%	0.0%	
Total	21392	21403	21323	21397	21351	21394	21390	149650
	14.3%	14.3%	14.2%	14.3%	14.3%	14.3%	14.3%	

Table 2.13: Observed Frequencies for the different ACCase and ALS amino acid substitutions among all plants after treatment in the greenhouse. The wildtype for every position is indicated in bold.

treatments in the greenhouse did not show any mutation. This value ranged considerably between products with 44% and 31% of the samples from Ralon Super and Axial showing no mutation, respectively. Only 5% of the plants surviving Focus Ultra in the greenhouse showed no mutation. Among the ALS-inhibitors 34%, 23% and 22% treated with 250g/ha, 500g/ha and 1000g/ha Atlantis WG showed no mutations while only 8% of the plants tested with MaisTer fluessig had no mutation present. A maximum of four loci were mutated per individual plant. This was however a combination of one mutation at the ALS enzyme and three at the ACCase enzyme. A maximum of three was observed for one enzyme separately (table 2.15). Between one and seven different loci had mutated per sample across all years with a median of four mutations per sample. This number was correlated with the average scaled infestation level over all years as calculated from section 2.2.5. The correlation was found to be low (r=0.17***) indicating that fields showing above average infestation do not contain more target site mutations.

Since treatment by the farmer in the field has an impact on the constitution of mutant genes in the population the treatment of the survivors in the greenhouse furthermore selects certain survivors based on the applied herbicide and dose rate (table 2.14 and table 2.15).

Table 2.14 shows that for the ALS enzyme most of the plants surviving a greenhouse treatment showed a target site at either position P197X or W574, while plants showing both target sites together were rare. The frequencies at which these occur varied among the different treatments applied but mutations at W574X were more often observed compared to mutations at P197X. An interesting aspect is that plants surviving the MaisTer fluessig treatment in the greenhouse show a significantly higher frequency of mutations at W574X compared to the treatment with Atlantis WG (Chi-Square: $\tilde{\chi}^2_{(3,\ N=3686)}$=1003.5, p<.001). This indicates that this mutation covers both Atlantis WG and MaisTer fluessig compared to P197X only covering Atlantis WG . Furthermore the frequency of plants showing no mutation (possibly EMR) is lower at the MaisTer fluessig treatment compared to the Atlantis WG treatments at the field rates (Chi-Square: $\tilde{\chi}^2_{(1,\ N=3686)}$=205.5, p<.001). The frequency of plants with no target site mutation decreases as the dose rate of Atlantis WG increases, indicating that plants with possible EMR are present for which a dose dependent function is usually observed. This suggests a stronger selection of EMR by low dose rates. Furthermore, a pre-selection with an ACCase-inhibitor in the greenhouse revealed fewer plants with a target site mutation than with selection by an ALS-inhibitor , indicating the selection pressure of the ALS-inhibitors . While no differences were observed for Ralon Super , Axial and the untreated control the frequency

of plants with no target site mutation was lower for plants preselected with Focus Ultra (Chi-Square: $\tilde{\chi}^2_{(9,\ N=9886)}$=262.5, p<.001) indicating a higher efficacy of this active ingredient on plants showing EMR. Survivors with no mutations after treatment with an ACCase-inhibitor were not assessed for EMR and cannot therefore be directly compared to plants with no mutation after ALS-inhibitor treatment as they survived a targeted application. For the target site mutations this comparison is possible.

Mutation	Atlantis WG 250g/ha	Atlantis WG 500g/ha	Atlantis WG 1000g/ha	MaisTer fluessig 1.5l/ha	Focus Ultra 2.5l/ha	Ralon Super 2.4l/ha	Axial 1.2l/ha	Untreated
no Mut	0.56	0.37	0.25	0.13	0.74	0.90	0.91	0.89
P197X	0.13	0.15	0.16	0.02	0.06	0.05	0.05	0.05
P197X, W574X	0.01	0.02	0.02	0.04	0.01	0.00	0.00	0.00
W574X	0.30	0.46	0.57	0.82	0.19	0.05	0.04	0.05
No.of samples	3045	2840	2754	798	673	7981	1227	55

Table 2.14: Selection by the different greenhouse treatments on mutations at the two loci assessed at the ALS enzyme

Furthermore the occurrence of mutations conferring resistance to ACCase-inhibitor represents the established ACCase resistance (table 2.15). Except for the pre-treatment with Focus Ultra in the greenhouse, the frequencies for plants without a target site mutation between the ALS-inhibitor and ACCase-inhibitor are within the same range. A mutation at position I1781X was the most frequently observed, followed by mutations at D2078X with all others seen less often. Plants pre-selected with Focus Ultra showed a considerably higher frequency for I1781X compared to treatments by Ralon Super and Axial which were both within the range of the untreated control Chi-Square: $\tilde{\chi}^2_{(3,\ N=9941)}$=1154.2, p<.001). All other mutations were of equal or lower frequency in samples pre-selected by Focus Ultra compared to the other herbicides sprayed. This implies that this mutation covers the entire range of the ACCase-inhibitor as Focus Ultra sharply selects for a mutation at I1781X. Plants with two or even three mutations do occur but with frequencies of <1%. Not all of the possible mutations were seen within plants with three mutations. For plants with two mutations all possible combinations of mutations were found.

Mutation	Atlantis WG 250g/ha	Atlantis WG 500g/ha	Atlantis WG 1000g/ha	MaisTer fluessig 1.5l/ha	Focus Ultra 2.5l/ha	Ralon Super 2.4l/ha	Axial 1.2l/ha	Untreated
no Mut	0.54	0.52	0.49	0.50	0.06	0.44	0.34	0.47
I1781X	0.15	0.16	0.17	0.18	0.59	0.14	0.19	0.20
I1781X, W2027X	0.02	0.02	0.02	0.02	0.08	0.02	0.03	0.00
I1781X, W2027X, I2041X	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
I1781X, W2027X, D2078X	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
I1781X, I2041X	0.01	0.01	0.01	0.01	0.04	0.01	0.01	0.00
I1781X, I2041X, D2078X	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
I1781X, I2041X,G2096X	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
I1781X, D2078X	0.02	0.01	0.01	0.01	0.04	0.02	0.03	0.00
I1781X, D2078X, G2096X	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
I1781X, G2096X	0.01	0.01	0.01	0.01	0.02	0.01	0.00	0.00
W2027X	0.09	0.10	0.10	0.11	0.02	0.13	0.14	0.04
W2027X, I2041X	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
W2027X, D2078X	0.01	0.00	0.00	0.00	0.01	0.01	0.03	0.02
W2027X, D2078X, G2096X	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
W2027X, G2096X	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.04
I2041X	0.05	0.06	0.06	0.06	0.01	0.06	0.04	0.04
I2041X, D2078X	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00
I2041X, G2096X	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
D2078X	0.06	0.06	0.06	0.05	0.10	0.11	0.16	0.18
D2078X, G2096X	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00
G2096X	0.04	0.04	0.04	0.04	0.01	0.04	0.01	0.02
No.of samples	3070	2866	2769	820	678	7981	1227	55

Table 2.15: Selection by the different greenhouse treatments for mutations at the five loci assessed at the ACCase enzyme

2.3.4 Population Analysis

So far, a characterization of samples that were collected in the field has been carried out. Samples were further analyzed in the lab and the efficacy ratings described and compared in the lab across crops, infestation levels and years. Now, the focus will be on describing the spatial and temporal patterns of resistance.

Spatial patterns of resistance were assessed by 1) relating genetic distance, expressed by pairwise Fst values at the ACCase enzyme to the geographic distance and 2) identifying clusters of fields with similar patterns regarding all the observations previously described.

For the first approach the frequencies of all alleles occurring at the ACCase enzyme were calculated. As was previously shown in table 2.15 the effect of Focus Ultra and other DIM herbicides needs to be considered as this product selects strongly for I1781X and controls potentially EMR resistant plants. These herbicides are used in dicotyledon crops and therefore only samples from winter and summer wheat, winter triticale, winter and summer barley and corn were considered for further analysis. The overall Fst value was 0.124. Calculated pairwise Fst values among all samples (regardless of whether they were from the same field but from a different year) ranged from 0 to 0.76, with a mean of 0.10 and median of 0.07. Mean values per field ranged from 0 to 0.26, with a mean of 0.04 and a median of 0.02. These values were considerably smaller than mean values from field comparisons alone, ranging from 0.03 to 0.49, with a mean of 0.09 and median of 0.08. This implies a smaller variation within samples from one field compared to samples from different fields. It also shows that an ACCase resistance pattern is rather fixed in the population. Values on a per farmer basis showed a range of 0 to 0.3 with a mean of 0.06 and a median of 0.05 and are therefore in between comparisons per field and across fields. It also shows that grouping by farmer has a smaller variation compared to totally random pairs. The genetic distance represented here is based not on neutral markers but on alleles identified as important for resistance. The data suggest the extent to which similar resistance patterns developed but cannot be used to estimate the relatedness of two samples. To assess for spatial auto correlation and identify possible distance effects, a Mantel Test was carried out showing no significant correlation between genetic diversity and geographical distance ($r=0.03$, $p=0.1$).

In a second approach, allele frequencies of all occurring alleles at every loci (2 ALS loci and 5 ACCase loci) were calculated. Survival rates for Atlantis WG (500g/ha) and Ralon Super (2.4l/ha) and infestation values were included for each field and year.

Using clustering with multiscale bootstrap resampling, the identification of fields with similar patterns at given confidence values was attempted. Fields clustered together indicate similarly progressed resistance and the location of clusters permits the visual identification of local patterns. Testing this multivariate set using MANOVA showed a barely significant interaction for Year and Crop (Test=Pillai, p=0.05). Values were thus normalized by mean and standard deviation for this interaction. Only fields with observations longer than one year and crops that had more than five observations in a given year were considered to avoid biasing by standardization (129 fields out of 1225 considered). The mean and standard deviation were calculated for all variables assessed to achieve a dimension reduction. Multi-scale bootstrap re-sample clustering was applied to identify clusters with high confidence. The clusters were divided into 95%, 90% and 80% confidence values identifying 9, 12, and 2 clusters respectively. Among these three Cluster intervals 32 (25%), 74 (57%) and 129 (100%) of the fields were assigned to groups (table 2.16). 8, 11 and 2 groups were formed for the 95%, 90% and 80% confidence intervals. As already shown by Fst highly similar fields exist and become clustered together, especially at higher confidence values. Graphical representation of these clusters showed again no spatial connection for Location Z and Location H as indicated by the 90% confidence value (figure (2.4). This indicates that fields which are highly similar to each other show no spatial pattern, with many fields not being assigned to any group. Location M showed very similar results on the first look but many fields were too different to be grouped into one of the extracted groups, indicating that this pattern cannot be generalized. Reasons for the similarity could be a similarity in farming style leading to similar selection pressure, a similar environment or both. The two clusters at the 80% accuracy interval mainly differ in the presence of mutations at W574 and the rate of survival, with Cluster A showing higher values compared to B, explaining why mainly fields from location M are included in this group. Finer scaled clustering reveals several other forms of clusters, including specific ACCase patterns that are not shown here. As clusters at 80% accuracy are mainly grouped generally by location, all of the fields of a given farmer were typically within one cluster. In the 90% and 95% group, farmers with more than one field in the analysis were not always grouped in the same cluster, indicating variation within the fields of individual farmers. This differs alone with the earlier analysis of the ACCase locus and is mainly driven by also considering the ALS enzyme. Different fields show different developmental stages to resistance to ALS-inhibitors , explaining the difference.

A Principal Component Analysis (PCA) approach on the dataset was attempted to find relationships between variables (not shown). However, the analysis by scree plot

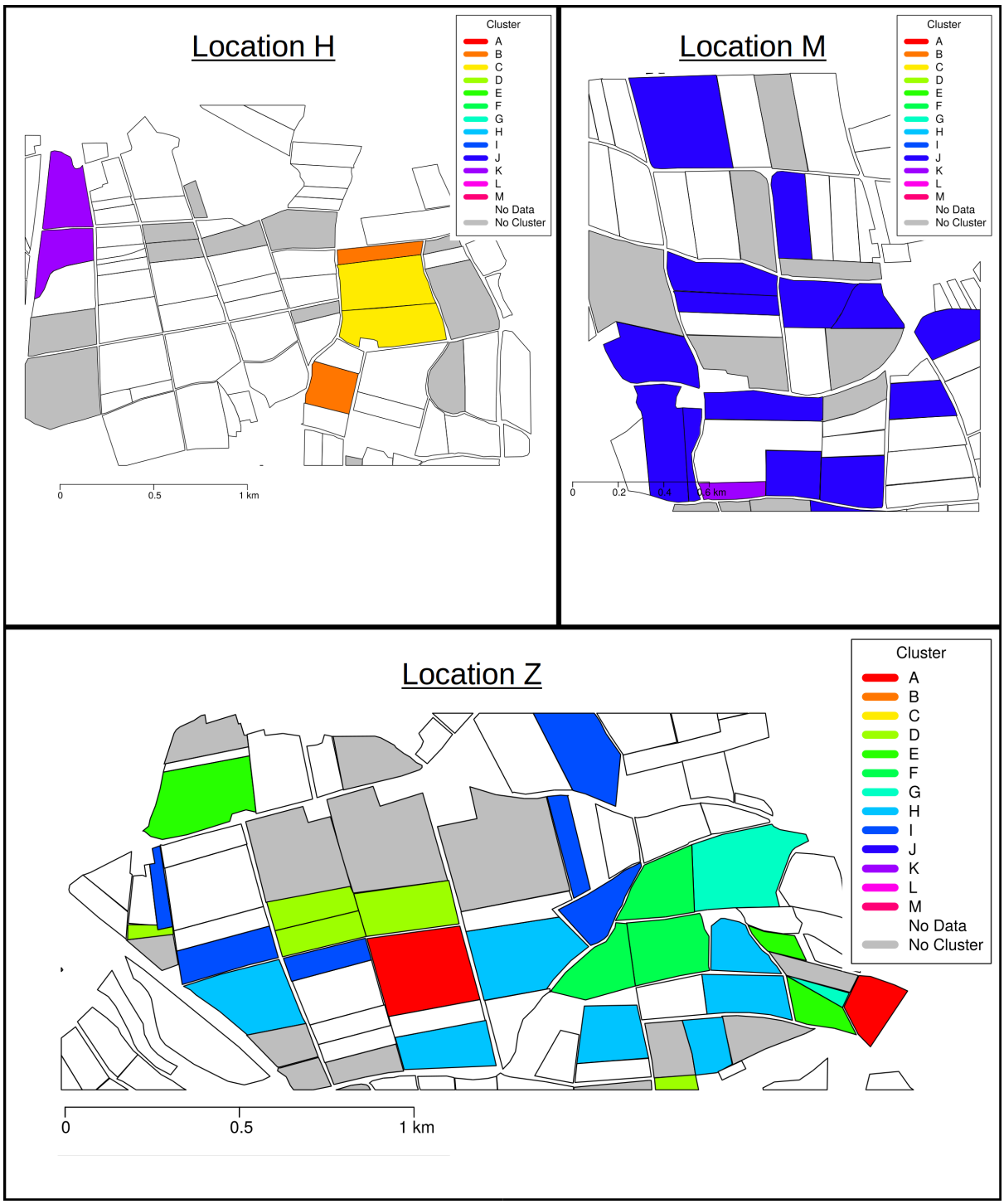


Figure 2.4: Closely related clusters (>90% AU) in color for the three sample locations. Grey color indicates fields that did not show high similarity to any other field and are therefore independent at the accuracy level assessed.

Location	Group	A	B	C	D	E	F	G	H	I	J	K	No Cluster
H		2	31										
M	Cluster80	25	4										
Z			67										
H			2	2		1		3				6	19
M	Cluster90					1					16	1	11
Z		2			8	4	3	4	13	7		1	25
H			2	2					6				23
M	Cluster95					2			1				26
Z		2			8		2	6	1				48

Table 2.16: Cluster Solutions based on Approximately Unbiased Cluster Solutions with 95%, 90% and 80% accuracy.

suggested to extract five components, which explain only 35% of the variance. This suggests that there is a lot of randomness in the dataset, leading to a lot of random resistance patterns, with none of the mutation patterns correlating highly with another. This conforms to table 2.14 and table 2.15 where almost all possible combinations of mutations are found. It also agrees with the findings of clustering and pairwise Fst analysis, since both also suggest the presence of an individual fingerprint in every field, distinguishing it from others.

Analysis of the homogeneity of fields over time was done by considering all variables assessed in the field, greenhouse and laboratory for each sample. The aim was to see whether two or more samples from the same field taken in different years/crops cluster together by considering the status of resistance to both ACCase and alis. Among the 156 fields assessed, there were only 19 fields grouped at least once in the same cluster conserved pattern found in multiple samples. Further analysis of these 19 fields revealed that 15 of these had high or very high average infestation levels of *A. myosuroides* with reduced or low efficacy of Atlantis in the greenhouse (table 2.17). This indicates that most fields with a temporally conserved pattern are fields with progressed resistance development. Among the four fields still with high efficacy to Atlantis WG one came from an organic farm and showed no resistance. Based on both resistance patterns and field observations, it was found that the pattern is still variable in a lot of fields over time. A selection of one point in time as a representative sample for an entire field might not give the full picture of the resistance status.

	Atlantis Control		
Infestation	High	Reduced	Low
Average	2	4	2
High	2		5
Very High			4

Table 2.17: Distribution of clustered fields over time relating to *A. myosuroides* infestation and control of Atlantis WG in the greenhouse.

2.4 Discussion

Management of *A. myosuroides* with selective grass active herbicides, such as ACCase-inhibitors and ALS-inhibitors is practiced primarily in the main cereal growing areas of western Europe. Reliance on these potent Modes of Action is very high, with increasing reports of resistance causing problems for farmers. While the ACCase-inhibitor family was introduced in the mid 1980s ALS-inhibitors were introduced in 1999 against grass weeds. This provides the opportunity to study two Modes of Action with a different use history. Genes and alleles involved in herbicide resistance are an interesting phenomenon as they are assumed to occur spontaneously and are enriched by the herbicide application performed by the farmer (Délye et al., 2013; Jasieniuk et al., 1996). Those that provide a fitness advantage in the event of selection are therefore enriched. The factors influencing selection are one of the questions that have been addressed in this study. It is also interesting to note that multiple genotypes (mutations) can result in the same resistant phenotype (resistant against a product in the field) (Délye et al., 2004; Baucom, 2016). Studies on the spread and development of resistance in *A. myosuroides* have so far focused only on cases with confirmed or suspected resistance. With regard to *A. myosuroides* these studies covered a broad overview of the diversity of resistance mechanisms and the distribution across a broad scale (Menchari et al., 2006; Knight, 2016).

Questions of the extent to which this is representative for the areas tested remain unanswered due to the missing randomness of the sampling. The potential extent of the resistance areas is furthermore unknown and proper risk analysis missing. This study was conducted to assess the abundance of resistance in three locations at a small scale with the intention of sampling an entire crop rotation of multiple fields from different farmers. Recorded observations from 1) different fields, 2) over multiple years , 3) in three different locations gave a unique set of data that can be used to describe the resistance situation on a small scale based on starting points with confirmed resistance. Observations included the field infestation at harvest, the remaining efficacy of seeds from field survivors, and the molecular characterization of resistance mechanisms, providing a significant level of detail. From these results further insights into the spatial and temporal dynamics of *A. myosuroides* resistance to ACCase- and ALS-inhibitors can be gained. Anderson et al. (2010) pointed out that many biological factors, like factors that influence the life cycle of a weed, occur at multiple scales. Small study areas, comprising field experiments or a few individual fields, can therefore be influenced by many factors outside the sampled zones. In terms of herbicide resistance this would mean that no real conclusion on (environmental) effects can be drawn if the sample size in a given location

is too small (chapter 3). Proper assessment of the collected samples prior to analyzing the causal underlying effects is therefore most critical. The studied region includes many small farms with 50-80ha of land per farm and intensive animal husbandry. This enables the study of numerous fields in a relatively defined environment at a very small scale, with high diversity in management practices due to the variety of farmers. This chapter should therefore serve as a basis to study and understand the causes of resistance that are considered in the following chapter. Here the focus is solely on status and changes of resistance in space and time in a small area based on studying all fields on a few km^2 for a complete crop rotation.
Key findings were:

- The overall infestation level at harvest and after selection by the farmer in the field was low.
- Resistance to the FOP/DEN class of ACCase-inhibitors was found in >80% of the analyzed samples while resistance to ALS-inhibitors at the field rates was found in <32% of the samples, with high variability between locations H, M and Z.
- Four patterns of resistance in survivors from greenhouse treatments were found.
  - There were two patterns in the survivors that could not be controlled with ALS-inhibitors in the greenhouse. One, covering both Atlantis WG and Mais-Ter fluessig was attributed to a mutation at W574L of the ALS-enzyme. The other, conferring only resistance to Atlantis WG was attributed to mutations at P197X or plants showing no mutation. Both patterns seem however geographically distinct, with mutations at W574L being predominant at one location and mutations at P197 at another.
  - For the ACCase-inhibitors there were also two distinct patterns. One covering Focus Ultra and the other covering all other chemical classes related to ACCase-inhibitors but not focus. A mutation at I1781X was found to be the cause for that. Both patterns occur in all locations analyzed.
- Spatial correlation was found to be low, indicating independent development of resistance from field to field.
- Temporal variability was high in fields where resistance to ALS-inhibitors is still in development. Most fields showing no temporal variability showed pronounced resistance to both MoA tested or were from organic farmland with no herbicide usage.

Infestation

All fields showed infestation with *A. myosuroides* at harvest time but not in all years indicating that *A. myosuroides* is present in all fields assessed. The low levels of infestation at harvest further indicates that farmers control *A. myosuroides* well and/or that the infestation prior infestation was low. However, hot spots of infestation could be identified both within and across locations. The higher infestation levels observed that were detected by standardizing infestation levels across years and crops clearly showed that the core fields belonging to location M had the highest infestation levels over the years. This can be either due to environmental factors or due to management aspects both comprising a favorable habitat for high population development. This higher level, in combination with the reduced efficacy of Atlantis on the observed field survivors, led to the conclusion that the resistance problem is the highest in location M. The lowest levels of infestation were found in Location H, with only a few fields showing high infestation levels not directly linked to a given farmer. Since multiple farmers are active in each location, farming practices might only be one indicator that can explain these differences. Kruecken (1975) established the link between soil attributes and infestation levels in his work on *A. myosuroides* . He found considerably higher levels of *A. myosuroides* on soils suffering, at least partially, from waterlogging. Menck (1968) was observing higher levels of *A. myosuroides* with higher levels of clay and loam in the soil. Both factors are given for the majority of fields in the study area according to farmers and are addressed in more detail in the next chapter (p. 86). *A. myosuroides* infestation also depends on yearly influences. Numerous studies have shown that several factors, among them germination and dormancy, differ a lot over time and so influence the *A. myosuroides* stand in the following season (Zwerger, 1993; Swain et al., 2006). Conditions over winter are another factor influencing the population of *A. myosuroides* (Kemmer et al., 1980). In the harvest season of 2012 there was an extremely cold period causing a lot of crop failure. Farmers were faced with the decision to plant a summer crop or leave the often heavily damaged winter crop, explaining the high levels of summer crops found for that particular year. Those who left the winter crop were often confronted with an uneven crop stand, giving the *A. myosuroides* the opportunity to develop a lot of tillers, which is reported to occur under favorable conditions (Menck, 1968). A general look at the sampled crops over time reveals that the majority of crops grown were all winter annuals. Due to their higher yield they are usually more favorable for the farmer than their spring counterparts but unfortunately have the same life cycle as *A. myosuroides* and so promote the weed (Lutman et al., 2013). Winter sown crops dominated not only this study area but also

the main agricultural land in Germany without specialty crops . Since the frost incident in 2012, an increased number of fields were sown with corn. Due to the restriction of certain insecticides used in oilseed rape the portion of corn has further increased recently, making it the only frequently observed summer crop in the study area. Studies from Kemmer et al. (1980) and personal observations show that low infestation levels of *A. myosuroides* in corn seem to produce less viable *A. myosuroides* seeds. Reasons for that are manifold e.g. decreased pollination due to missing partners and a pronounced self incompatibility in *A. myosuroides* (Moss, 1983). Competition with the crop and the resulting harvest that starts 5 months after seeding causes additional pressure on *A. myosuroides* to rapidly reproduce. However, the late seeding date in the season, usually at the end of April/beginning of May, is the biggest reason why the infestation levels of *A. myosuroides* are lower in this crop than in others (table 2.5). The seed quality in terms of germination rate and vigor is typically also lower in samples taken in corn than samples from a winter wheat field, for example (personal observations). Legumes are not frequently observed because the area is dominated by farms focused on animal husbandry, preferring crops with high fertilizer input.

Analysis of samples in the greenhouse and laboratory

Seed assessment was done on samples that were mostly pre-selected by the farmer in the field with different ALS-inhibitors and ACCase-inhibitors . With winter triticale, winter wheat and corn this is typically done using ALS-inhibitors . The observed control rates at the greenhouse level for the ALS-inhibitors are therefore usually lower in these crops as a result of the pre-selection by the farmer. Levels for the ACCase-inhibitors Ralon Super and Axial remained low across all crops and infestation levels, highlighting the established resistance pattern to both of them. An exception to that was cycloxydim (Focus Ultra) which typically retained high efficacy in the greenhouse. However, as this product cannot be used in cereals it is only applied in oilseed rape and pulses, which explains the trend that the observed efficacy of cycloxydim in the greenhouse in oilseed rape is lower (table 2.3). This is attributed to a target site mutation at I1781X which is reported to confer resistance to the complete set of ACCase-inhibitors (Powles and Yu, 2010). While mutations at D2078 were also reported to cover the entire range of ACCase-inhibitors , its presence in this study is lower. A potential fitness cost involved with that mutation might be the reason for this (Menchari et al., 2007a). Another difference was detected in the efficacy of the two ALS-inhibitors tested. The main reason identified for the higher efficacy of MaisTer fluessig was its control of P197X, while mutation W574L confers resistance to both ALS-inhibitors tested. Interestingly, this mutation was more

pronounced at location M, where it was found in the majority of fields. Traces were also seen in Location Z but here the mutation P197T dominated (Appendix, p. 165ff). While MaisTer fluessig is exclusively marketed for corn in Germany, corn is not the explanation for these findings as corn cropping increased in all three locations after 2012 and cases with high levels of W574L were found after application of another ALS-inhibitors namely Atlantis WG . Given we assume a random and spontaneous occurrence of mutations, the question of why the local distinction between these two mutations exists remains (Jasieniuk et al., 1996). Fitness attributes that differentiate the two mutations as a cause of isolation-by-environment (IBT) are one potential explanation that needs further research.

The variety of mutations occurring generally showed that the full set of mutations reported for *A. myosuroides* was present in the data (Powles and Yu, 2010; Délye et al., 2004). There were also some new mutations that were currently only reported for other species (e.g. I1781V, I1781T). All these mutations survived a selection by the farmer in the field and consecutive greenhouse testing with commercially available herbicides. This shows that intensively sampled populations on a small scale of a few km^2 show the same broad spectrum of resistance mechanisms that were previously only found with studies of larger often country wide areas (Menchari et al., 2006; Knight, 2016). The complete set of mutations reported in these studies was therefore already present at the beginning of the selection process and is therefore part of the standing genetic variation of *A. myosuroides* (Délye et al., 2010b). Sampling of further areas might therefore reveal an even larger set of potential mutations conferring a resistant phenotype. Laboratory testing for TSR mutations thus allows further differentiation of resistance causes which cannot be done in the greenhouse. The diversity on the plant and field level was found to be very high with up to four mutations occurring per plant and fields containing mutations at all loci so far reported for *A. myosuroides* . This is more than Marshall et al. (2013) reported per individual plant and is due to the fact that samples were taken randomly and continuously for most of the fields, meaning a larger number of combinations were selected. This diversity is not surprising as Délye et al. (2010b) reported a high genetic diversity of genes and alleles enabling *A. myosuroides* to survive a herbicide treatment and leading to a high genetic redundancy. It also shows that many genetic patterns occur that result in the same phenotype (phenotypic convergence, Baucom (2016)). Since early phases are the most crucial for the final pattern of resistance, the unique combination of an established ACCase-inhibitor resistance with an ALS-inhibitor resistance in development gives the opportunity to study the outcome of a past selection process by the farmer

(ACCase-inhibitor resistance) and a present ongoing selection (ALS-inhibitor resistance).

Sampling precision of mutations occurring at low frequencies remains problematic however. Assessment of resistance is only an indirect assessment of the soil seedbank based on field survivors. Therefore minimal enrichment that might or might not become a problem for the farmer is observed in one year but not the next. An example was given in table 2.12 where a strong selection towards survivors of Atlantis WG was found for "Z014" in 2013. These survivors were selected with an ALS-inhibitor prior to 2011 but were only found in low numbers in 2011 and not at all in 2012. This indicates that mutations occurring at low levels, but slightly higher than the natural frequency, have a good chance of being missed, especially with the low infestation levels observed in most fields.

Comparison with other studies on occurrence of resistance

Surveys of resistance to either ACCase-inhibitors and/or ALS-inhibitors have previously been conducted for different areas.

Resistance to ACCase-inhibitors was assessed in various studies by collecting fields either randomly and non-randomly. In a recent study from the UK Knight (2016) found resistance to ACCase-inhibitors in 90% of the samples. Selection of cases in his study was however not random, making a sampling bias possible. However, the resistance levels found for ACCase-inhibitors are comparable, with regard to FOP/DEN, with the data presented here. They are also comparable to data presented by Menchari et al. (2006) who found high levels of ACCase resistance in the majority of samples following farmer complaints. Resistance to Ralon Super was also found in all 10 of the samples tested from Germany by Petersen et al. (2012). In a survey of 19 randomly collected fields Moss et al. (2014) found resistance in 100% and 58% of the cases for clodinafop (similar active ingredient as Ralon Super) and cycloxydim (active ingredient in Focus Ultra) respectively. Resistance to cycloxydim is therefore more abundant in the UK samples assessed by these authors than in Hohenlohe. Resistance to FOPs are however at comparable levels. In a comparison of non-randomly collected samples across Europe Délye et al. (2011) found that resistance to fenoxaprop (Ralon Super) was expressed in almost all the samples. Only samples from Turkey and the Netherlands were still found to be sensitive in the majority of cases (80% and 43% respectively). Results between non-random and random samplings for resistance to ACCase-inhibitors did not reveal differences in the abundance of resistant cases (Moss et al., 2014). This is, for the FOP/DEN but not DIMs, in agreement with the findings of this study as resistance to these two chemical classes was found in all samples. This indicates that in cereal

dominated crop rotations, resistance to FOPs is established.

Menchari et al. (2006) found that I1781L, the predominant mutation in this study, was also the predominant mutation in France. Predominant occurrence of I1781L was also seen in other samples from France and the UK (Marshall et al., 2013; Délye et al., 2007). It indicates that the DIM class was widely applied which furthermore leads to the conclusion that oilseed rape or other dicot crops play an important role in wide parts of UK. Samples taken from these crops by Knight (2016) strengthen that hypothesis. As the DIM herbicides are only used in specific crops and not in cereals, the higher abundance of samples resistant to DIMs can be explained by differences in the crop rotations between Hohenlohe and the UK. Despite TSR, EMR to ACCase-inhibitors was found as the major resistance reason by Délye et al. (2010b).

Resistance to Atlantis WG was found by Petersen et al. (2012) in 20% of samples tested from Germany. Knight (2016) found resistance to Atlantis WG in over 90% of the samples analyzed. Both studies are however not representative as they were taken from samples of suspected or confirmed resistance. In a sampling by Chauvel et al. (2006) resistance to flupyrsulfuron, an ALS-inhibitor , was found in 98% of the populations with 50% of the analyzed plants being actually resistant. Hull et al. (2014) found that among 122 non-randomly selected fields 75% showed resistance against Atlantis WG . In a survey of 19 randomly collected fields Moss et al. (2014) found resistance to Atlantis WG in 47% of the tested cases. These randomly collected samples show that the level of resistance to Atlantis WG in the UK is more progressed compared to the 32% found in this study. For resistance to ALS-inhibitors Moss et al. (2014) found that two mutations (P197T, W574L) or EMR occurred in the samples tested. This was also found by Hull et al. (2014) for their samples from the UK. However, in this study no sample showed resistance to Atlantis WG solely based on EMR.

Obtaining reliable data for the actual development of resistance is difficult as most studies focus on previously confirmed resistance cases overestimating the situation. However, all studies found resistance to the FOP class of the ACCase-inhibitors for the main cereal growing areas in Europe. Therefore it can be assumed that this is widely established. While in the UK TSR seem to play a major role, EMR seems more important in the rest of Europe with regard to ACCase-inhibitors . Reliable data for the ALS-inhibitors is rare but results from this study suggest that compared to the UK the levels of resistance found were lower. Furthermore, EMR was found in a lot of samples in the UK whilst it was not widely pronounced in the samples of this study. In addition, non-random sampling was found to overestimate the resistance situation to the ALS-

inhibitors . It needs to be highlighted that most of the studies compared here based their observations on non-randomly selected fields. The sampling bias becomes most evident when comparing the 47% of resistant cases found for Atlantis WG using random sampling from Moss et al. (2011) to the non-random data obtained at 75% and 90% Hull et al. (2014) and Knight (2016). The data of this study found 32% of the fields showed low efficacy for the field rate of Atlantis WG though it should be emphasized that most of the fields showed infestation at low levels. The samples in which high infestation levels occur together with low remaining herbicidal efficacy in TRZAW, TTLWI or ZEAMX are even lower (5% of the analyzed samples). Therefore phenotypical resistance at the field level was not observed in a lot of samples that showed reduced efficacy in the greenhouse. More reliable randomly collected data is necessary to accurately predict the development status of ALS-inhibitors as this is missing today. If the focus is to accurately describe the resistance situation to a MoA not being considered widely resistant, random sampling is necessary. Data represented here accurately represents the situation in an area with confirmed resistance cases to two MoA. The data is comparable with other studies regarding resistance to FOP/DEN but shows lower levels of resistance to DIM. The overall level of resistance to ALS-inhibitors was found to be much lower compared to other samples. There are numerous potential reasons for this, including a sampling bias in non-random observations or different management regimes and environmental conditions.

One question remaining is that of how farmers facing pronounced resistance to ACCase-inhibitors manage to achieve low infestation levels in e.g. HORVW or BRSNW. The main reason is the use of other herbicide families such as pre-emergent compounds like Flufenacet (HRAC-MoA K3) or Isoproturon (HRAC-MoA C2).

Population Analysis

Data of the overall Fst values fits well with observations by Menchari et al. (2006) who sampled a larger geographical range. Pairwise Fst comparisons of samples taken from the same field showed lower fst values than the average across all comparisons. As pairwise Fst values were calculated for alleles occurring at the ACCase enzyme, disregarding samples that were potentially selected with a DIM herbicide, this data again represents the already well established resistance to ACCase-inhibitors in the study area. More importantly, the long selection period with this MoA created a unique fingerprint for each field that is highly stable over the years. One needs to stress here that Fst values were calculated from mutations conferring resistance and not from neutral parts of the genome.

Therefore these values do not then represent a kinship of populations, but rather a similarity of resistance mechanisms that have evolved and were compared across different

fields and multiple samples from the same field. Other authors analyzing neutral parts of the genome found low genetic diversity across different populations which is not surprising for an allogamous species (Chauvel and Gasquez, 1994; Menchari et al., 2007b). However, both Chauvel and Gasquez (1994); Menchari et al. (2006) showed that field borders are the boundaries of spread. This is an important finding as it offers a basis for farmers to successfully apply a weed resistance management regime to their field without a high influx of resistance from neighboring fields minimizing their efforts. This was addressed in this study for the first time for neighboring fields at several locations with answers to these points generated by multiple analyses. The first focused solely on ACCase-inhibitors as resistance, in particular to Ralon Super and Axial which has been shown to be well established. Independent of the actual herbicide application by the farmer in the sampling year, resistance was found in almost all fields reporting low remaining efficacy in >80% of the samples. We can therefore conclude that in most of the several hundred seeds banks checked, almost every seed is resistant to Ralon Super and Axial. The individual resistance structure (genotypes) explaining these resistant phenotypes is however attributed to a unique combination of target-site mutations and EMR. Therefore pairwise Fst values were calculated based only on these five SNPs . Values were low but still distinguishable between fields. No spatial correlation was found as tested by the Mantel Test leading to the conclusion that neighboring fields distinguish from each other as much as fields that are far apart based on a resistance pattern that was selected for not more than 20+ years after the introduction of the first ACCase-inhibitors . It also revealed that this pattern is very homogeneous as mean Fst values within a field over multiple years were smaller than average Fst values of neighboring fields. Furthermore, average values per farm range between averages of individual fields and averages of any random pair of fields. This in addition highlights that through their management regime a farmer may apply a selection pressure that differs considerably from their neighbor. Similar observations were reported by Aper et al. (2012) who also showed that *Chenopodium album* occurring on one farm is more related than that of other farms. These authors linked this to seed transport. There are however several points related to *A. myosuroides* that would contradict that and are in favor of the hypothesis that similar environments with similar selection pressure (farmer) give the same results. First, transport through combine use is observed, though most of the *A. myosuroides* seed is shed at harvest and therefore not taken up (Menck (1968) and personal observations). The seeds that are taken up are likely to be less fertile as they develop last and are therefore not well pollinated (Moss, 1983). Second, transport through the use of other machinery is possible but the likelihood

of that is also small given that one farmer's fields do not necessarily border each other meaning that in driving on roads to access other fields, tires are potentially rid of seed. With seeding or tillage equipment it is a little different but the amount of seed attaching to the machines can be neglected and might only play a role for seed distribution within a farm. Resistance occurrence therefore might also depend on the stability of the system and the number of seeds that it can buffer before a severe accumulation by the management applied in the field occurs. Farmers with a higher diversity in crop management might fight these seeds using measures other than herbicides preventing accumulation over time. Farmers with more susceptible systems are prone to that effect, though the risk of selection of resistance in the designated field without the introduction is already higher than for those applying resistance management. Third, spreading through manure is less likely as grass seeds die rapidly in both biogas fermenters and manure (Schrade et al., 2003).

In a second approach all variables assessed in the field, greenhouse and laboratory were standardized across years and crops and grouped by hierarchical clustering via multiscale bootstrap re-sampling based on average mean and standard deviation per field. The clustering of data taken from various sources has not been done before. Clustering has been previously carried out for genetic data, survey data and other multivariate observations (Menchari et al., 2006; Küpper et al., 2016). Here, the results of field infestation levels, remaining efficacy of field survivors in the greenhouse and the frequency of target site mutations in the samples are considered. This is one method used to detect borders of spread or similar habitats in landscape genetics (Anderson et al., 2010) The analysis also shows that the groups formed show no spatial structure (figure 2.4) based on approximate unbiased p-Values at the 95% and 90% level. Minor cases of local groups are mostly due to farmers dividing one field into two for some years but farming them together in others. Therefore these cases were recorded as being two individual fields.

In summary, both analyses revealed independent resistance evolution from field to field. The variability observed is due to random chance, as no relationship between the occurring mutations was found between fields. This agrees with findings by (Menchari et al., 2006; Baucom and Mauricio, 2007; Okada et al., 2013) who also revealed no relationship between fields at larger scales. Délye et al. (2015) found meanwhile that the easy distribution of locally developing resistance alleles is the main driver in the spread of resistance at a local scale for *Senecio vulgaris*. Délye et al. (2010a) also identified high gene flow between neighboring fields as likely to spread resistance in *A. myosuroides* . The authors linked this to the high pollen cloud (source) that reached the low number of

A. myosuroides plants in the organic field (sink). They concluded that sufficient control of *A. myosuroides* by a farmer does not prevent resistance from occurring in his field, as it might be introduced by a neighboring field. Findings were backed up by the authors showing similar patterns in the analysis of neutral markers between those fields. These results are contradictory to the results presented here. With regard to the genetic diversity it needs to be stated that the overall genetic diversity among populations of *A. myosuroides* is high and several authors were not able to distinguish even those populations far away from one other (Menchari et al., 2007b; Chauvel and Gasquez, 1994). Furthermore, the level of infestation will need to be higher in the conventional fields than it would in an organic field. This was not observed among the organic fields sampled however (data not shown). To fully elucidate that point neutral marker analysis of an entire set of fields per region needs to be carried out with fields chosen from different farmers. This will enable proper assessment of the total genetic diversity occurring at a given location.

Variability over time with all site-years individually assessed revealed that among the 13% of fields grouped together at least once, most fields had an already pronounced phenotype to both MoA tested. Another field was an organic field. The remaining 87% of the fields showed no similarities between the different samples taken from different years. This does not contradict the findings carried out by assessing pairwise Fst values against ACCase-inhibitor only as the variability comes from resistance to ALS-inhibitor is not yet fully established. It reveals some fields as having less infestation due to ALS-inhibitor being applied in one year with higher infestation when ACCase-inhibitor was applied to an already ACCase-inhibitor resistant population the year after. As farmers are mostly aware of the reduced efficacy of ACCase-inhibitor they are mostly used in combination with other products to achieve good herbicidal control. While a single application of ALS-inhibitor in one year might give good control in the field, all the survivors at the field level might be resistant, indicating a case of very sharp selection pressure in the field (2.12). 16 out of the 19 fields with already reduced variability were thus fields where the selection by or the absence of ALS-inhibitor generated an already fixed resistance pattern as observed when considering pairwise Fst values for the ACCase mutations alone.

This reveals that individual fields undergo various selection pressures over the crop rotation where *A. myosuroides* is usually selected by different MoA. A constant crop rotation with different selection pressure in different years applied will therefore produce different results within the developmental phase of resistance but ultimately result in a stabilized system in time, presenting a unique fingerprint for that field. In other words

one could have the impression that a farm specific selection pressure (the management by the farmer) applied to a macro habitat (field) leads to a very specific resistance pattern that is almost unique for every field. Specific environmental factors (soil) that shape the population of *A. myosuroides* on a field level in the absence of gene exchange are the main reason for that. As a *A. myosuroides* population always consists of seeds from multiple generations (Naylor et al., 1972) and resistance to ACCase-inhibitors was found in almost every plant, it can be assumed that the speed of selection of resistance to ALS-inhibitors is accelerated as plants with this trait are also propagated in the absence of the selection pressure. A high *A. myosuroides* population resulting from resistance to ACCase-inhibitors can therefore boost the propagation of resistance to ALS-inhibitors explaining simulation results by Bagavathiannan et al. (2014).

Conclusion

This study distinguishes itself from other studies analyzing spread of resistance, e.g. Délye et al. (2004), Menchari et al. (2006), Baucom and Mauricio (2007), Délye et al. (2015), Okada et al. (2013), in that it studies neighboring fields that are randomly and for the most part repeatedly sampled based on three initial starting points. While the starting points were not randomly chosen, all of the fields surrounding them can be considered to have been chosen at random. As stated earlier, the infestation level of *A. myosuroides* and the crops that were assessed enables the drawing of applied conclusions of the resistance situation at a local landscape level. It needs to be emphasized that this is considered pro-active research on resistance, since most of the farmers contacted did not observe problems with the control of ALS-inhibitors, the MoA being introduced at the latest for selective grass control in cereals. In addition, most of the fields showed infestation levels below that which is considered to cost yield, meaning farmers typically had *A. myosuroides* under control or had low levels of infestation before applications. This allows an assessment of resistance development at an early phase, before a major outbreak, a period highly critical in the development of resistance (Neve et al., 2009). Pro-active measurements are important as Collavo et al. (2013) and Rummland et al. (2014) have shown that a certain frequency of accumulated resistance does not go away and so the accumulation of the resistance genes and alleles needs to be avoided in the first place. This completes the observational part of the thesis and leads consequently to the analysis of key selection factors that are related to the observations of this chapter.

Chapter 3

Analysis of Field History and Soil Information to Determine the Driving Factors behind Herbicide Resistance to ALS-Inhibitors in *Alopecurus myosuroides* Huds.

3.1 Introduction

Chapter 2 analyzed the resistance status to ACCase- and ALS-Inhibitors in *A. myosuroides* at various scales. There are 1) the three different locations, 2) multiple neighboring fields at every location in each area representing a complete picture of the location and 3) various sample years representing the development of resistance over time. This unique set of data has not been collected before and enables insights into the true underlying factors of resistance. It was found that resistance develops individually on the field level and exchange with neighboring fields is low. Resistance to ACCase-inhibitors was found in every field while resistance to ALS-inhibitors was found to be under development and not yet established in the seedbank . This is an important starting point as it enables us to assess the evolution of resistance in action for ALS-inhibitors . As the choice of fields was random, the evaluation of factors between resistant and sensitive fields in a defined area can give insights into their true importance. The chosen approach of sampling fields over multiple years instead of carrying out plot experiments was found to be more suitable for resistance development. Reasons for that were discussed earlier (section 1.2.4). The main points to consider in the analysis of resistance development are:

1. **Adequate sample size.** Resistance development depends on both frequency of resistance genes and the mutation rate (Jasieniuk et al., 1996). The latter is believed to be fixed and the frequency of resistant genes will therefore be determined by the available number of plants. In early developmental phases, which are observed for resistance to ALS-inhibitors here, having an adequately large and similar environment (field) is particularly essential for having a reasonable chance to observe this early phases of resistance development.

2. **Diversity in Management** is given by assessing multiple farmers with different types of farms using different crop rotations. As multiple fields per farmer were usually sampled, assessment of the same management on different fields and evaluation of its outcome is possible.

A. myosuroides management options and their impact on the population were described earlier (section 1.2.1). Here the focus is on studies relating management factors to resistance. Most studies into herbicide resistance have focused only on describing the pattern of resistance occurring without considering its causes. Among those who did, the studies can be separated into field surveys (Beckie et al., 2008; Evans et al., 2016; Knight, 2016) and field trials (Rummland, 2015). Beckie et al. (2008) found that the

amount of cereals in the crop rotation and the diversity of the crop rotation are important in describing differences between ACCase resistant *A.fatua*. Knight (2016) also linked the resistance situation to the amount of spring crops based on fields samples for *A. myosuroides* resistance to ALS-inhibitors . Herbicides as the main selection pressure were furthermore seen as influential in the development of resistance (Evans et al., 2016). Délye et al. (2010b) was able to show that the overall amount of herbicide applications considering all MoA was not significantly different between resistant and sensitive cases, while the amount of ACCase-inhibitors being used was directly linked to the resistance level to ACCase-inhibitor found. The authors concluded that resistance is directly linked to the selection pressure occurring at the field level. Modeling studies on the other hand were able to highlight the importance of the herbicide regime, though they often lacked validation. Bagavathiannan and Norsworthy (2014) showed in a simulation study that a lack of herbicide rotation brings about rapid resistance increase to ALS- and ACCase-inhibitors in *Echinocloa crus-galli*. The authors linked the rapid spread of resistance to a second MoA with the failure to stop using an already resistant MoA soon enough after the first resistance symptoms. An increase in the seedbank size enriched with resistance to both MoA results from that. Moss (1985) found big differences in the number of seeds in the seedbank size ranging from <10000 seeds to >50000 seeds per sqm. This large range might be attributed to the *A. myosuroides* control achieved in the field but also to the soil attributes on the corresponding fields. The relationship between soil data and both management and resistance has yet to be established. This question is important as there might be differences in the output of the same measure in different environments. However, individual factors have so far only been assessed separately, without looking at the whole picture and may not have had large sample sizes with regard to the area assessed to clearly separate and generalize findings among the factors under consideration. Field trials on the other hand might only be able to characterize strategies that measure the effect of several management factors on one or a few populations. They are set up as controlled environments that represent the factors at the location in which they are conducted. This creates the difficulty of drawing larger conclusions as a lot of the "noise" that might be caught by random sampling of fields cannot be assessed to create stronger statements.

The open questions in this section are therefore:

- Is the environment where fields were sampled homo- or heterogeneous between and within individual fields? Is it likely that other factors besides management play a role in resistance development and need to be considered in further research? In

sampling the fields it became obvious that *A. myosuroides* occurs in some fields in a very patchy manner which could not be related to spraying patterns. This was also observed by Balgheim (2009). The question is therefore whether fields can be grouped into those with a lower and higher abundance of *A. myosuroides* which directly influences the initial frequency of resistance. The hypothesis is therefore that the same management practice may work on some soils and fail on others. The carrying capacity for *A. myosuroides* might therefore be important for some management systems.

- What type of crop management systems occur in the study area and how can they be related to resistance? The characterization of such systems and how they are distinct from each other would be key in the establishment of a proper ranking of resistance factors. Based on established studies it can be assumed that herbicide use frequency is a key factor in the establishment of resistance. Despite this, crop rotation plays the most important role. As crop rotation is directly connected to the possible herbicide regime these factors are not completely independent of each other. Tillage on the other hand seems to be related to the individual preference of the farmer based on own observations. Therefore the impact of this factor will need to be viewed in light of the system the farmer applies. The management systems identified will than be linked to the overall resistance status in the field.

It is assumed that based on the number of farmers involved, multiple different farming systems might be extracted from the data. It is furthermore assumed that the landscape is homogeneous, though with enough variance to explain local differences that occur. The combination of the soil data and farming data can create a great number of possible combinations that occur in the fields and therefore explain the observed individual resistance development.

3.2 Material & Methods

3.2.1 Soil Map Analysis-Derivation of Variables

A soil map from the area of interest was obtained from Reg (2015) in form of an ESRI-Geodatabase. The geodatabase consists of a map with different soil classes and their corresponding soil attributes. 1006 fields were analyzed by their soil properties. The number includes fields that were sampled in chapter 2 but also fields that belong to known farmers but were not sampled. The additional fields were considered to get a very broad overview of the region and its underlying soils. 86 different soil classes were found within the 1006 fields. The area of most fields was composed of more than one soil class, with one field containing seven (table 3.1). Every soil class contains various attributes that characterize the soil at a given location (Appendix 7.1). The coverage of every soil class on each field was calculated using R and the packages raster (Hijmans, 2015a), sp (Roger S. Bivand and Gómez-Rubio, 2013), rgeos (Bivand and Rundel, 2015) and maptools (Bivand and Lewin-Koh, 2015). Soil classes with less than 5% coverage in one field were excluded and the coverage of the remaining soils adjusted to 100% coverage. This was done to account for small deviations from the exact field boundaries as the overlaying shapefile holding the fields was made manually. For soil attributes with alphanumerical values or categorized data, a conversion to numerical values based on data provided in the literature or the map accompanying manual was conducted. Unless otherwise stated numerical data were obtained from Reg (2015). For soil texture the mean, minimum and maximum values were calculated while for other parameters the calculation of only minimum and maximum values was done.

Soil Classes Per Field	Number of Fields
1	114
2	353
3	322
4	163
5	50
6	3
7	1

Table 3.1: Soil homogeneity of individual fields represented by the number of soil classes found per field.

Soil Texture variables were created based on alphanumerical input and derived from table 3.2 for the different soil types. Values for the different soil types, describing the

texture of the soil, were derived from Eckelmann (2006). As multiple soil types were given per soil class averages, minimum and maximum values were calculated. Six values were derived to numerically describe the soil type of a given soil class. Clay_min and Silt_min present the lowest value of clay and silt that was found for the range of soil types listed for the given soil class. Clay_max and Silt_max present the maximum value. In conjunction, both groups display the range of either clay or soil for the given soil class. Clay_mean and Silt_mean present an average of all soil types within a soil class.

Soil Type	Clay_mean	Clay_min	Clay_max	Silt_mean	Silt_min	Silt_max
Ls2	21	17	25	45	40	50
Ls3	21	17	25	35	30	40
Ls4	21	17	25	23	15	30
Lt2	30	25	35	40	30	50
Lt3	40	35	45	40	30	50
Lts	35	25	45	23	15	30
Lu	24	17	30	58	50	65
Sl2	7	5	8	18	10	25
Sl3	10	8	12	25	10	40
Slu	15	12	17	45	40	50
Tl	55	45	65	23	15	30
Ts2	55	45	65	8	0	15
Tu2	55	45	65	42	30	53
Tu3	38	30	45	58	50	65
Tu4	30	25	35	68	65	70
Uls	13	8	17	58	50	65
Ut3	15	12	17	78	65	90
Ut4	21	17	25	74	65	82

Table 3.2: Soil types and corresponding mean, min and max values for clay and silt characterizing each soil type. Mean values were derived from the mean between min and max values

pH-Value characterizes the soil pH-value. Minimum and maximum values for every soil class were calculated using table 3.3. This value can be influenced by the farmer and therefore provides only an estimate.

KF-Value characterizes the water permeability through the first meter of soil under water saturated conditions. Minimum and maximum values for every soil class were calculated using table 3.4.

Soil Organic Matter (SOM) characterizes the soil organic matter content [%]. This might vary over time and therefore be only an estimate of the real situation. Minimum

PH Class	PH_MIN	PH_MAX
8	7.0	8.0
7	7.0	7.0
6b	6.5	7.0
6a	6.0	6.5
5	5.0	6.0
4	4.0	5.0
3	3.0	4.0

Table 3.3: pH-Value Classes as extracted from the soil map with minimum and maximum pH values for every soil class

KF Class	KF_MIN	KF_MAX
1	0	1
2	1	10
3	10	40
4	40	100

Table 3.4: Kf-Value Classes as extracted from the soil map with minimum and maximum values for every class in cm/d

and maximum values for every soil class were calculated using table 3.5.

Soil Depth (GRUND) characterizes the mechanical soil depth before reaching a soil layer [cm]. Minimum and maximum values for every soil class were calculated using table 3.6.

Field Capacity (FK), Usable Field Capacity (UFK) and Cation Exchange Capacity (KAK) were present in a numeric form with minimum and maximum values provided in the shapefile. Therefore no further transformation of this data was carried out.

Statistical analysis of Soil Parameters

To identify similar groups of soils in order to reduce the 86 different soil classes a cluster analysis was carried out. All variables created were scaled prior the analysis by mean and

SOM Class	SOM_MIN	SOM_MAX
2	1	2
3	2	4
4	4	8
5	8	15
6	15	30

Table 3.5: SOM classes as extracted from the soil map with minimum and maximum values for every class in %

GRUND Class	GRUND_MIN	GRUND_MAX
1	0.00	1.40
2	1.50	3.00
3	3.10	6.00
4	6.10	10.00
5	10.10	100.00

Table 3.6: Soil depth classes as extracted from the soil map with minimum and maximum values for every class in cm

standard deviation to account for different measurement units. KMeans clustering was applied using the kmeans function in R (R Core Team, 2016). K groups were identified by plotting the sum of squares within groups against the number of clusters extracted using the Elbow criterion. The area spanned by every cluster among the 1006 fields sampled was calculated using the areaPolygon function of the geosphere package (Hijmans, 2015b). A Principal Component Analysis (PCA) was carried out to identify features in the data set that were used to describe the groups identified by clustering. PCA was carried out using the psych package (Revelle, 2015). An assessment of variables was done prior to PCA, with highly correlating variables (r >0.9), which are thought to bias the analysis, excluded. This resulted in only the maximum value for every soil attribute being considered and the minimum value being discarded besides for KAK where the correlation between minimum and maximum value was <0.9. PCA assumptions were checked as suggested by Field et al. (2012) using Bartlett's correlation test to assess for sufficient correlation between variables, the Kaiser-Meyer-Olkin (Kaiser, 1974) measure of sampling adequacy, and the det function to test for singularity of the input data. Based on the Kaiser-Criterion components with and eigenvalue >1 were extracted. PCA was carried out using orthogonal rotation (varimax). Components were described based on variables loading high on them. Plotting of PCA loadings by cluster was done using the ggplot2 package (Wickham, 2009). After characterizing the soil groups using PCA a prediction of soils from all soil variables reported using random forest was attempted. The random forest algorithm was trained using the random forest package in R (Liaw and Wiener, 2002). 5000 trees were developed with a random sample size of 51 and a final node size of 1. Out of the box sampling accuracy was used as an indirect measure for sampling accuracy as further testing data was not available.

3.2.2 Field History Analysis

Data collection

Field history information was collected by interviewing farmers managing the fields described in chapter 2. There was an attempt to obtain information on crop rotation, tillage and herbicide use for the past 10 years. However not all farmers were able to provide all this information, for various reasons. In some cases, additional information about fertilizer regime, crop varieties and yield were collected. Since only a small number of farmers were able to provide this data, it was not included in the final analysis. Overall sufficient information on the important farm management factors, reviewed by Lutman et al. (2013), were collected. These decide the population dynamics and are also found to be important in explaining resistance evolution of *A. myosuroides* . A ranking of crop competitiveness of different varieties was not possible, due to lack of information about that varietal feature provided by farmers.

Data preparation

As resistance towards ACCase-inhibitors has been proven to be established, the term resistance in this chapter will only refer to the status towards ALS-inhibitors unless specified otherwise. The period of selection for ACCase-inhibitors was prior to the 10 years assessed here, starting in 1980 with DIM and FOP herbicides in dicot crops. The use was extended in 1990 with fenoxaprop and clodinafop being used together with a safener in wheat and triticale.

Based on the observations in chapter 2, a classification of the resistance status for Atlantis WG was derived and fields grouped into Resistant (R), developing resistance (I) and Sensitive (S). To achieve these observations of infestation level, scaled infestation level, Atlantis WG control in the greenhouse, the occurrence of P197X, W574X and EMR and the likely sprayed Mode of Action (MoA) were taken and clustered together. In the absence of clear groups that were not found in the previous chapter, an attempt was made to group the fields based on the phenotypical resistance status in the field and infestation levels found across multiple years with the help of these clusters. As resistance definition is always based on seed collections it will never accurately reflect the true resistance status in the soil seed bank. To circumvent the problem several patterns across observations were identified using clustering and used to derive the resistance categories R, I and S.

The MoA likely sprayed was coded as 1 (ALS-inhibitor use likely) and 0 (ALS-inhibitor use unlikely). The aim of this cluster analysis was to identify patterns in the seed samples

occurring after the selection by the farmer in the field. Only fields with at least two observations were included in the analysis. Clustering was done using "manhattan" distance and the "ward.D" method of the function "hclust" of R. The data was scaled by mean and standard deviation prior to clustering to account for different scales. The analysis revealed seven groups that are presented with their corresponding (non scaled) averages (table 3.7). An example of an interpretation of the groups would be as following:

- Group 1: The samples came from a field that was most likely sprayed with an ALS-inhibitor (e.g. crop=TRZAW, ZEAMX), the infestation was low and below average for this crop in the sampling year as indicated by the values for infestation and scaled infestation. Control by Atlantis WG was still high and low frequencies for the assessed TSR mutations were observed in field survivors. Therefore, the samples were regarded as **S.**

- Group 2: The samples came from a field that was most likely sprayed with an ALS-inhibitor . Infestation was high and above average. However, the control of Atlantis WG in the greenhouse was still high and little or no plants with target-site mutations were found. Therefore, these samples were regarded as **S.**

- Group 3: The samples came from a field that was most likely not sprayed with an ALS-inhibitor (eg. BRSNW, HORVW). The infestation was low and below average. Control by Atlantis WG was still high and low frequencies for the assessed TSR mutations were found. Therefore, the samples were regarded as **S.**

- Group 4: The samples came from a field that was most likely not sprayed with an ALS-inhibitor (eg. BRSNW, HORVW). Infestation levels were high and above average for the crop in the sampling year. While control by Atlantis WG in the greenhouse was still high, few survivors were found that carried a target site mutation or showed metabolic resistance. These findings, in the absence of a selection by the farmer and together with the high infestation levels (different to group 3) led to the grouping of these samples as **I**.

- Group 5: Samples in this group came from either crop and showed slightly elevated infestation levels that were still below average for the corresponding crop in the sampling year. However, low control by Atlantis WG was observed and a high frequency of Enhanced metabolic Resistance (EMR) and/or target site resistance (TSR) detected. The dominating target-site mutation was often W574X. Therefore these samples were regarded as **R**.

- Group 6: Samples were most likely sprayed with an ALS-inhibitor . The infestation was high and regarded as above average for the crop in the sampling year. The remaining efficacy of Atlantis WG was found to be low, with a large number of plants showing a target site mutation (W574X). The samples were regarded as **R**.

- Group 7: Samples in this group showed high and above average infestation levels, with low control of Atlantis WG in the greenhouse and a high frequency of TSR (Pro197X). These samples were regarded as **R**.

Only fields with multiple observations were considered, with the majority of counts for a given group giving the classification. An example of this would be the classification of a sample into I, R, R for 2011, 2012 and 2013 respectively. The final grouping of this sample would then be R. In case of a draw, the more severe category got priority (e.g. R over I or I over S). Since the analysis of target-site mutations was missing for some samples, the classification was done based on crop, infestation level and Atlantis WG control in the greenhouse. This is justifiable, as table 3.7 shows that the classification of S, I and R can be derived from this if necessary.

Group	PP-Group	Infestation	Scaled Infestation	Atlantis Control (%)	P197X (%)	W574X (%)	EMR (%)	Rating
1	1	1.00	-0.81	87.81	4.54	2.42	10.11	S
2	1	2.72	0.59	87.98	2.41	4.44	6.52	S
6	1	3.57	1.17	13.11	7.41	73.03	8.73	R
7	0/1	3.17	0.94	37.58	63.92	2.75	12.33	R

Table 3.7: Resistance Classification into R, I, S for individual observations from chapter 2. PP-Group indicates the likely herbicide MoA applied by the farmer before the sample was taken. 0=ALS-inhibitor probably not sprayed and 1=ALS-inhibitor use likely. Values for Infestation indicate the infestation level from 0 to 5, scaled infestation indicates the normalized infestation levels (mean=0)

The analysis of resistance causes focuses on the data from the years 2005–2010 for the R group, and 2007–2013 for the I and S samples. A different time frame was analyzed for R in order to avoid bias, as farmers in this group reported having changed their crop management as a reaction to resistance. This was not the case for I and S and therefore a more recent time frame was analyzed for these groups. This allowed the analysis of

a greater number of samples since not all farmers were able to provide field history information from 2004/05 on. Data from 2012 were excluded since it was not considered representative due to the long frost period with crop failure and resulting summer crop seeding.

To analyze the data in a quantitative form, several indices were calculated (table 3.8). These numerical values allowed the application of statistical tests as data obtained from farmers was in plain text. Variable formation was done based on literature review and experimentation.

Variable	Explanation
WCereals	The proportion of winter cereals in the crop rotation
SCereals	The proportion of summer cereals in the crop rotation
WCrops	The proportion of winter crops in the crop rotation
SCrops	The proportion of summer crops in the crop rotation
NCrops	Number of different crops used (winter wheat, triticale and spelt were counted as one)
DicotCrops	The number of dicot crops in the crop rotation
Corn	The amount of corn in the crop rotation
SeedingDate	The proportion of delayed seeding events in the crop rotation. Early, average and late seeding dates for the various crops were determined together with a local crop advisor
Ploughing	The proportion of ploughing in the crop rotation
ALOMYHerb	The number of herbicide applications against *A. myosuroides* divided by the number of years observed
Herb_App	Total number of herbicide applications in the crop rotation divided by the number of years observed
Molecules	The number of different active ingredients applied
GrpB_Products	The number of different GrpB-Products applied in the crop rotation
UniqueMoA_Grasses	The number of different Modes of Action used against *A. myosuroides*
ALOMYHerbGrpB	The number of ALS-Inhibitors (HRAC Group B) divided by the number of years observed
GrpG_App	The number of Glyphosate Application in the crop rotations
GrpA_App	The number of ACCase application in the crop rotation
Flufenacet	The proportion of Flufenacet (HRAC K3) used against *A. myosuroides* . Only flufenacet was considered as it is the only preemergent compound being used against *A. myosuroides* . Other preemergent products are considered in UniqueMoA, Molecules and Herb_App

Table 3.8: Abbreviations and Description of Variables created to analyze field management data. Where not otherwise indicated occurrences were counted and divided by the number years analyzed (crop rotation = 6yrs)

Statistical Analysis

Spineplots and cluster analysis were done with R 3.2.4 (R Core Team, 2016). Pairwise comparisons of the different Atlantis Status were carried out using Fishers Exact Test for count data. PCA and Kmeans analysis was conducted as described in section 3.2.1.

All maps in this section were drawn using the packages raster (Hijmans, 2015a), sp (Roger S. Bivand and Gómez-Rubio, 2013), rgeos (Bivand and Rundel, 2015), maptools (Bivand and Lewin-Koh, 2015), geosphere (Hijmans, 2015b), mapplots (Gerritsen, 2014) and maps (Becker et al., 2016).

3.3 Results

3.3.1 Soil Data

As a first step in the analysis of key factors related to resistance development, soil attributes were analyzed to characterize the study area. Table 3.9 gives a broad overview of the distribution of the various soil parameters assessed in the study area. Clay values ranged from 11.8% to 55.0% depending on the soil class. Mean and median values for clay_min of about 21% indicate that the area generally contains lots of heavy soils. Field capacity ranged from 40l/m^2 to 600l/m^2. The depth of soils was also very variable, ranging from 1.5dm to >10dm. However, the median of soil depth (GRUND_MIN) was 10dm which indicates that most fields had adequate depth. While some soils were high in organic matter (>10%) the median was between 2-4%. Water permeability varied greatly, between <1cm/d and >100cm/d indicating the presence of soils where water was unlikely to penetrate quickly into deeper layers and which are therefore prone to water logging.

Variable	Min	Q25	Mean	Median	Q75	Max
clay_max	17.0	24.0	31.7	31.0	37.2	55.0
clay_mean	15.0	19.6	26.8	26.5	31.7	46.5
clay_min	11.8	14.6	21.3	21.0	25.9	37.5
silt_max	47.0	60.8	69.6	70.6	77.5	90.0
silt_mean	39.7	54.1	61.6	63.2	68.0	78.0
silt_min	31.7	46.9	52.7	55.0	57.5	65.0
FK_MAX	100.0	380.0	400.0	395.0	440.0	600.0
FK_MIN	40.0	220.0	274.4	295.0	350.0	460.0
GRUND_MAX	3.0	53.0	76.2	100.0	100.0	100.0
GRUND_MIN	1.5	6.6	8.6	10.1	10.1	10.1
SOM_MAX	3.0	4.0	4.9	4.0	6.0	19.0
SOM_MIN	1.5	2.0	2.4	2.0	3.0	9.5
KAK_MAX	40.0	300.0	318.9	320.0	350.0	430.0
KAK_MIN	20.0	150.0	206.0	210.0	257.5	380.0
KF_MAX	1.0	5.5	24.4	25.0	40.0	100.0
KF_MIN	0.0	0.5	6.0	5.5	10.0	40.0
NFK_MAX	70.0	140.0	156.6	155.0	190.0	240.0
NFK_MIN	20.0	70.0	95.0	90.0	120.0	160.0
PH_MAX	4.5	6.2	6.6	6.5	6.8	8.0
PH_MIN	3.5	5.5	5.9	5.8	6.5	7.0

Table 3.9: General distribution of soil parameters. Min= minimum value, Q25= 25% quantile, Q75= 75% quantile, Max= maximum

Kmeans clustering revealed eight relevant soil groups among the 1006 fields assessed

which were extracted by analyzing the attributes for the 86 soil classes discovered in the study area (table 3.10). The two biggest soil groups (Cluster 1 and Cluster 6) in the area covered 2221ha and 1387ha respectively, representing 73% of the assessed area. The number of fields in which Cluster 6 was identified (1030) is larger than the 1006 fields assessed which is due to the multiple soil classes that can occur within the same field. The same also applies for other soil groups.

Cluster	Area Covered [ha]	Fields/ part of Fields Covered
1	1386.80	786
2	135.70	102
3	9.60	5
4	75.90	51
5	480.30	454
6	2221.00	1030
7	318.80	178
8	108.60	79

Table 3.10: Area [ha] and number of fields spanned by the different clusters extracted from kmeans analysis

To identify the characteristics of the soil groups a PCA was carried out with orthogonal rotation (varimax). The Kaiser-Meyer-Olkin measure gave a value of 0.66, verifying the sampling adequacy. Bartlett's test of sphericity ($\tilde{\chi}^2_{(45)}$=552.4, p<0.01), indicated a sufficiently large correlation between variables (Field et al., 2012). Based on Kaiser's criterion three components were extracted. The three Principal Component (PC) with an eigenvalue >1 extracted give a total variance of 75% (PC1=39%, PC2=21%, PC3=16%). The items that group on the component suggest that component 1 represents wetness and nutrient retention ability of a soil (table 3.11). Samples scoring high on this component usually have high field capacities, high KAK and low Kf-values without restriction in depth. Component 2 represents the workability (heaviness) and water availability of the soil . Values scoring high on this component have high silt values and higher usable field capacities which is favorable for agriculture. The opposite, values scoring low on component 2 represent former or actual grassland fields with high clay content and high SOM. Component 3 represents the alkalinity level of soils as indicated by the score of PH_MAX, with a higher score indicating higher pH values but also often lower Kf and clay content.

Plotting the clusters by their respective scoring on the three components enables characterization of each cluster (figure 3.1). Clusters 1, 2 and 8 load highly overall on PC1, indicating that these clusters represents highly fertile but wet soils that also show

Variable	PC1	PC2	PC3
clay_mean	0.26	**-0.70**	**0.56**
silt_mean	0.28	**0.59**	**-0.44**
FK_MAX	**0.93**	0.04	0.11
GRUND_MAX	**0.73**	0.43	-0.08
SOM_MAX	0.35	**-0.63**	-0.13
KAK_MAX	**0.92**	-0.13	-0.04
KAK_MIN	**0.90**	-0.02	-0.17
KF_MAX	**-0.52**	0.19	**0.50**
NFK_MAX	**0.52**	**0.76**	0.07
PH_MAX	0.00	-0.07	**0.89**
Eigenvalue	3.86	2.06	1.61
% of variance	39	21	16

Table 3.11: Variable loading on the three components extracted with corresponding eigenvalues and variances explained. Factor loading with $>|0.5|$ are highlighted in bold

no restrictions in soil depths. The opposite is true for Cluster 3 and 4, which are often shallower and do not show such high water levels. Clusters 1, 5 and 8 are soils that are preferred for agriculture due to the reduced clay content and a higher usable field capacity as indicated by the loading on component 2. Clusters 2, 3, 4, 6 and 7 are soils with high clay content (heavy soils) with higher SOM. Cluster 2 in particular loads highly on component 2, indicating former grassland use. Soils in Cluster 4, 5 and 7 show high pH values while soils in cluster 3, 6 and 8 are more acidic.

Based on soil properties, a prediction of soil classes can be attempted. This is interesting as it enables us to derive the same groups from data of other regions which can be important in comparing locations in terms of their likelihood of showing resistance. A prediction of the soil clusters using the random forest approach leads to a classification accuracy of 85% meaning that the correct cluster could not be determined for only 15% of the 86 soil classes. This needs to be further tested, as a subset of the training data set not used for the development of an individual tree was used for cross validation. The confusion matrix of the algorithm is given in table 3.12 showing the frequency to which a certain group was correctly detected and, in case of false classification, which group was predicted instead. It can be seen that clusters 1 and 6 were mostly predicted correctly. The two soils grouped into cluster 3 showed an 100% classification error. However, the soils were classified as cluster 6 which shows similar characteristics to cluster 3 (figure 3.1). The very accurate prediction of cluster 2, which was identified as representing former grassland fields, is also important.

Comparisons between the map with the distribution of the eight soil clusters (figure

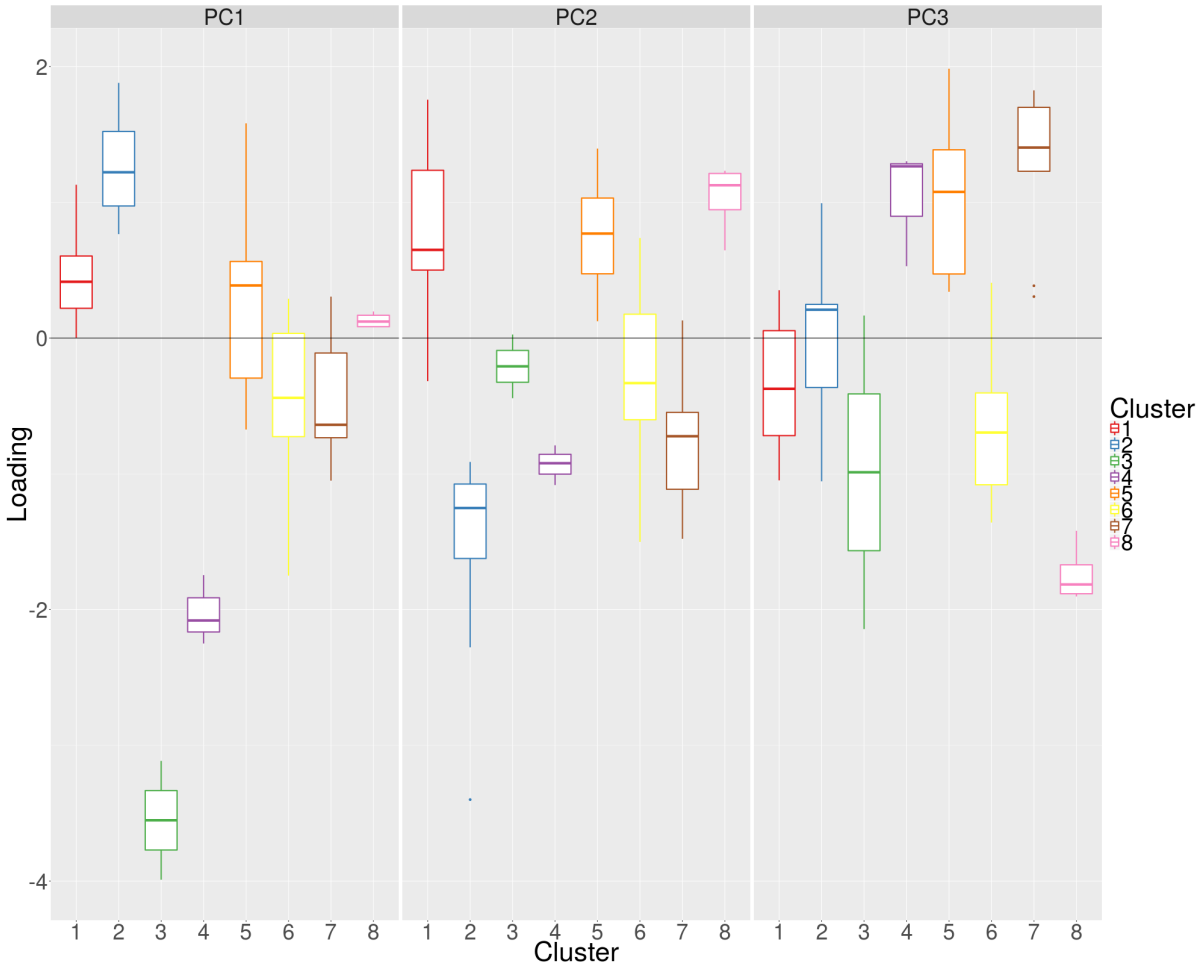


Figure 3.1: Distribution of soil groups (clusters) among the three components analyzed by PCA

Cluster	1	2	3	4	5	6	7	8	Error (%)
1	19	0	0	0	1	0	0	0	5
2	0	9	0	0	0	0	1	0	10
3	0	0	0	0	0	2	0	0	100
4	0	0	0	1	0	0	2	0	67
5	1	0	0	0	11	1	1	0	21
6	0	0	0	0	0	19	1	0	5
7	0	1	0	0	0	1	7	0	22
8	0	0	0	0	0	0	0	4	0

Table 3.12: Confusion matrix showing the classification of the prediction of every cluster based on the soil attributes provided with the corresponding classification error

3.2) and the map with normalized infestation values (figure 2.2, p. 30) indicate that many fields showing high infestation levels occur in soil clusters 2 and 5. However, all areas are dominated by clusters 1 and 6 as table 3.10 suggests. Cluster 4 seems to occur exclusively in location Z and contains fields that show some resistance development (Appendix 7.2, 165ff.). However efforts to directly link soil data to infestation levels or other observed attributes from chapter 2 failed (data not shown). The reasons for this are 1) farmers manage these soils differently which makes separation between management and soil effect difficult and 2) many fields consist of multiple soils and soil clusters at varying coverages which makes the analysis of the effect of one soil cluster difficult.

3.3.2 Field History Data

Variables for non-chemical measures were assessed and compared between the different Atlantis statuses as defined in table 3.7. The difference between S/I and S/R but not I/R was always significant (α=0.05) for variables WCereals, SCereals and NCrops. Significant differences between states of resistance were found for WCrops and SCrops across all comparisons (table 3.13), however these two factors are highly linked with each other as a farmer can only grow either a winter or summer crop. This indicates that the fields classified as resistant had fewer summer crops and more winter annuals but also lower crop diversity as indicated by the lower number of different crops planted (NCrops) compared to S. Fields classified as S and I showed higher frequencies of corn than R fields. In addition S fields were more frequently ploughed than R fields. Delayed seeding was seen less in R than in I and S. Generally no difference was found for the number of dicot crops in the rotation among all combinations assessed.

With regard to the variables assessed for herbicide application, no significant difference was found for Molecules, GrpB_Products, UniqueMoaGrasses, GrpG_App, GrpA_App (table 3.14). This indicates that the variety of products and MoA used does not vary between the groups. Furthermore it implies that glyphosate use is a special case that cannot be related to a specific resistance status but must be seen within the management system it occurs. In terms of treatment frequency, differences were detected between ALOMYHerb, HerbApp and ALOMYGrpB with a significantly higher treatment frequency observed for R fields compared to S. Significant differences between S/I were only seen for ALOMYGrpB and between I/R for ALOMYHerb. Fields classified as resistant were furthermore more frequently treated with flufenacet than sensitive fields. In general S fields were sprayed less frequently with herbicides and received fewer applications against *A. myosuroides* compared to the resistant fields. Intermediate fields were less fre-

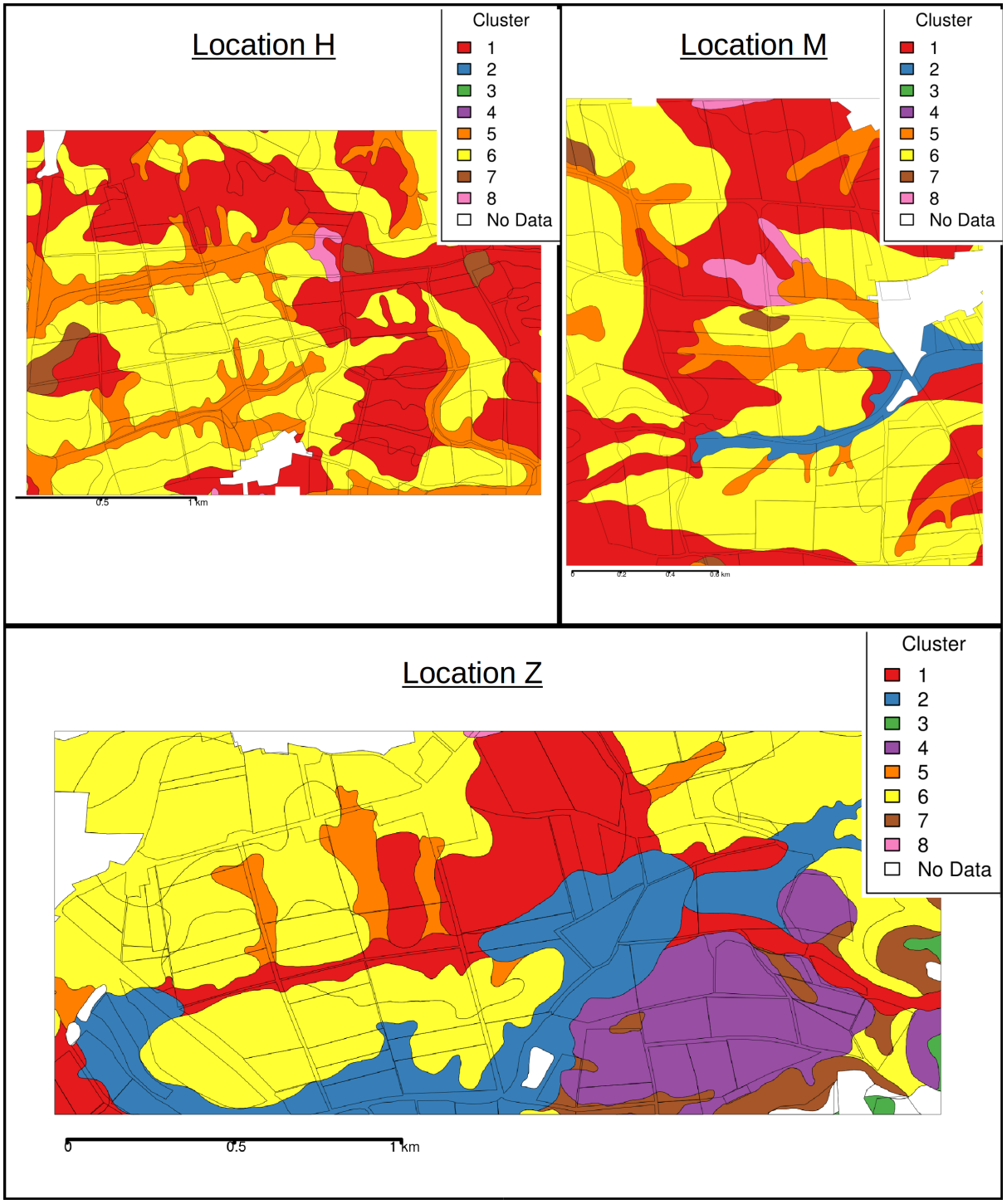


Figure 3.2: Maps of the eight soil clusters extracted using Kmeans clustering and their distribution in the core fields of location H, M and Z

Variable	Atlantis Status	Frequency among the 6yrs assessed							Sum	Pair	p-Value
		0/6	1/6	2/6	3/6	4/6	5/6	6/6			
WCereals	I			1		13	10	1	25	S/I	0.00
	R				1	12	20	2	35	S/R	0.00
	S			4	15	26	6		51	I/R	0.35
	Total			5	16	51	36	3	111		
SCereals	I	24		1					25	S/I	0.00
	R	31	3	1					35	S/R	0.00
	S	25	17	7	2				51	I/R	0.38
	Total	80	20	9	2				111		
WCrops	I					3	9	13	25	S/I	0.02
	R					1	5	29	35	S/R	0.00
	S				5	18	16	12	51	I/R	0.03
	Total				5	22	30	54	111		
SCrops	I	14	8	3					25	S/I	0.01
	R	30	3	2					35	S/R	0.00
	S	12	15	19	5				51	I/R	0.03
	Total	56	26	24	5				111		
Corn	I	19	4	2					25	S/I	0.35
	R	34	1						35	S/R	0.04
	S	39	3	6	3				51	I/R	0.02
	Total	92	8	8	3				111		
DikotCrops	I	4	13	8					25	S/I	0.85
	R	3	23	8	1				35	S/R	0.10
	S	10	23	18					51	I/R	0.54
	Total	17	59	34	1				111		
NCrops	I			2	17	5	1		25	S/I	0.03
	R			4	28	2	1		35	S/R	0.00
	S			2	19	26	4		51	I/R	0.19
	Total			8	64	32	6	1	111		
Ploughing	I	4	4	8	4	3	2		25	S/I	0.73
	R	10	9	10	2	2	2		35	S/R	0.04
	S	8	6	9	8	12	6	2	51	I/R	0.55
	Total	22	19	27	14	17	10	2	111		
SeedingDate	I	7	13	3		2			25	S/I	0.21
	R	28	7						35	S/R	0.00
	S	22	15	8	4	2			51	I/R	0.00
	Total	57	35	11	4	4			111		

Table 3.13: Distribution of non-chemical management data with total sums of observations and the corresponding Atlantis Status. Pair indicates the corresponding variable groupings (Comparison) for Fishers Exact Test of count data and are displayed together with the corresponding p-values

quently treated with herbicides compared to R but the intensity of ALS-inhibitor among the *A. myosuroides* treatments was comparable to the resistant fields.

Combined Analysis of Soil and Management Data

Soil and management data are not independent of each other and were therefore analyzed together. To assess the connection between these two and their influence to resistance, a PCA was conducted using all initially created variables for both soil and management data. The PCA was carried out to identify common features in the data that were then used to define similar groups of management using clustering. This identifies management systems that are subsequently related to the observed resistance status.

To meet the PCA requirements the variables WCrops, NCrops and GrpB_Products were excluded as either their share of common variance was too low or their correlation with other variables was found to be too high for PCA. Testing for sampling adequacy of the PCA using the Kaiser-Meyer-Olkin yielded a value of 0.6 which is above the 0.5 suggested by Kaiser (1974). Bartlet's test of sphericity ($\tilde{\chi}^2_{(136)}$=987.1, p<0.01) indicated sufficiently large correlations in between variables (Field et al., 2012).

Based on Kaiser's criterion five components were extracted explaining 68% of the total variance (table 3.15). Variable grouping on these factors suggest that component 1 represents the intensity and diversity of chemical weed control. Component 2 represents the degree of corn farming. High levels of corn with frequently observed delayed seeding and higher usage of glyphosate (GrpG_App) are typical for fields showing high scores on this PCA. Higher intensities of ALS-inhibitors against *A. myosuroides* are furthermore observed. In addition less use of flufenacet and less dicot cropping is observed. Component 3 represents the amount of IWM being used. Variables including Ploughing, DikotCrops, SCrops but not Corn load highly on that component while high reliance on ALOMYGrpB and high shares of WCereals are not observed for fields scoring high on this component. Component 4 represents the soil attributed tillage pattern. On fields scoring high on this component ploughing is mostly done in with lighter soils that are not prone to water logging and is avoided on heavier and sometimes also shallower soils. Component 5 indicates wet and heavy soils as indicated by the loading of Soil Cluster 2 on this component.

Six groups of similar management forms were identified using KMeans analysis. Scores of these six groups generated from 106 fields on the components from the PCA

Variable	Atlantis Status	Frequency among the 6yrs assessed								Sum	Pair	p-Value
		0/6	<2/6	<4/6	<6/6	<8/6	<10/6	<12/6	>12/6			
ALOMYHerb	I			1	8	6	9	1		25	S/I	0.09
	R				6	12	7	10		35	S/R	0.00
	S			4	14	25	7	1		51	I/R	0.03
	Total			5	28	43	23	12		111		
Herb_App	I				1	10	9	3	2	25	S/I	0.72
	R				2	9	7	11	6	35	S/R	0.03
	S				6	14	22	6	3	51	I/R	0.22
	Total				9	33	38	20	11	111		
Molecules	I				1		5	4	15	25	S/I	1.00
	R				1		6	7	21	35	S/R	0.82
	S				1	2	9	7	32	51	I/R	1.00
	Total				3	2	20	18	68	111		
GrpB_Products	I		2	15	5	3				25	S/I	0.09
	R		2	15	13	5				35	S/R	0.46
	S		5	19	24	3				51	I/R	0.49
	Total		9	49	42	11				111		
UniqueMoaGrasses	I		2	16	4	3				25	S/I	0.10
	R		4	17	12	2				35	S/R	0.19
	S		4	34	13					51	I/R	0.34
	Total		10	67	29	5				111		
ALOMYGrpB	I		6	15	4					25	S/I	0.03
	R		7	17	7	4				35	S/R	0.00
	S		27	21	3					51	I/R	0.40
	Total		40	53	14	4				111		
GrpG_App	I	19	6							25	S/I	1.00
	R	27	8							35	S/R	1.00
	S	39	12							51	I/R	1.00
	Total	85	26							111		
GrpA_App	I	3	8	13	1					25	S/I	0.84
	R	2	14	14	5					35	S/R	0.18
	S	5	20	25	1					51	I/R	0.42
	Total	10	42	52	7					111		
Flufenacet	I	9	16							25	S/I	1.00
	R	7	25	3						35	S/R	0.03
	S	19	32							51	I/R	0.19
	Total	35	73	3						111		

Table 3.14: Distribution of herbicide management data with total sums of observations and the corresponding Atlantis Status. Pair indicates the corresponding variable groupings (Comparison) for Fishers Exact Test for count data and are displayed together with the corresponding p-values.

Row	PC1	PC2	PC3	PC4	PC5
WCereals	-0.05	-0.28	**-0.78**	0.13	0.20
Corn	-0.09	**0.86**	-0.08	0.00	-0.03
SCrops	-0.04	**0.65**	**0.48**	0.09	-0.14
DikotCrops	0.21	**-0.53**	**0.49**	-0.24	-0.15
Ploughing	0.16	0.04	**0.65**	**0.47**	-0.03
Herb_App	**0.92**	0.08	0.02	-0.03	-0.02
Herbicides	**0.77**	0.08	-0.01	0.08	0.20
ALOMYHerb	**0.81**	-0.26	-0.16	-0.06	-0.05
ALOMYGrpB	0.39	0.25	**-0.73**	0.03	-0.29
UniqueMoA_Grasses	**0.59**	-0.24	0.07	0.16	0.28
GrpG_App	**0.40**	**0.57**	0.12	-0.25	0.15
GrpA_App	**0.55**	-0.36	0.17	-0.28	0.00
Flufenacet	0.28	**-0.44**	-0.05	0.30	0.35
SeedingDate	-0.15	**0.71**	0.08	-0.01	0.16
Soil2	0.23	0.19	-0.05	0.12	**0.64**
Soil4	0.06	0.02	0.01	**-0.79**	0.15
Soil6	-0.00	-0.03	0.01	0.33	**-0.76**
Eigenvalue	3.47	3.03	2.15	1.33	1.55
Variance explained %	20	18	13	8	9

Table 3.15: Variable loadings of PCA results for soil and management data based on 106 fields. Factor loading with $>|0.5|$ are highlighted in bold.

are shown in figure 3.3. The groups are described as follows:

- **Cluster 1** shows low scores on PC1 but higher scores on PC3 (figure 3.3). This indicates that herbicide use in these fields is less intense and various non-chemical tools are applied. Cluster 1 additionally shows lower values on PC4 indicating that these fields are less frequently ploughed.
  Most of the fields in this cluster are characterized as sensitive.

- **Cluster 2** shows elevated levels on PC1 and the highest values on PC3 indicating high herbicide diversity and intensity with use of non-chemical measures in combination. These fields are mostly on the better soils that are less prone to water logging.
  Most of the fields from this cluster are characterized as sensitive.

- **Cluster 3** shows high levels of herbicide intensity combined with lower values on PC3 (IWM) and the lowest values on PC4. Weed management is done through high herbicide intensity and ALS-inhibitor application. Winter cereals dominate the crop rotation but diversity is higher compared to cluster 4+5. These fields are less frequently ploughed as they are often quite shallow but also prone to water

logging as indicated by the high scores on PC5.
Most of the fields in this cluster are S followed by fields being classified as I.

- **Cluster 4** is very similar in management to cluster 3 but features the wettest and heaviest fields among those assessed. They have no restriction in depth.
  Most of the fields in this cluster are classified as being R or I.

- **Cluster 5** scores lower in terms of herbicide diversity and number of applications but features very simplified crop rotation with high shares of winter cereals and reliance on ALS-inhibitors for *A. myosuroides* management. Soils are mostly lighter compared to clusters 3 and 4.
  Most of the fields are classified as R or I.

- **Cluster 6** represents fields in the corn rotation with no resistance occurring in that fields. Corn fields show higher values for late seeding events as the sowing date after corn is late as a results of the late harvest.
  Most of the fields in this cluster are S

Clusters show differences in the occurrence of R, S and I Clusters 1, 2 and 6 show a lower occurrence of resistant fields compared to cluster 4 and 5 (table 3.17). No difference in between clusters 4 and 5 and between clusters 3 and 4 were found. In addition clusters with lower counts of resistant fields (1, 2, 6) did not differ from each other in the occurrence of R, S and I. Values for the interaction of cluster and Atlantis Status were analyzed by ANOVA for Infestation Values and by MANOVA for Greenhouse data (Control by Atlantis WG and MaisTer fluessig) and laboratory data (EMR, P197X and W574X), respectively. Not all fields were tested in the laboratory and so different values between NTotal and N as indicated in table 3.16 occur. Both ANOVA and MANOVA revealed significant differences for the three different Atlantis Status tested but neither the cluster itself nor the interaction of cluster and Atlantis Status was found to be significant (α=0.05, data not shown). This means that the different agricultural practices identified led to significant differences in the occurrence of resistance, but not to a distinct resistance pattern that would be unique to the management and soil characteristics for a given cluster. The greater abundance of fields carrying a P197X mutation observed in Location Z and a W574X mutation at Location H could not be explained with differences in management as fields of all three locations can occur within the same management cluster.

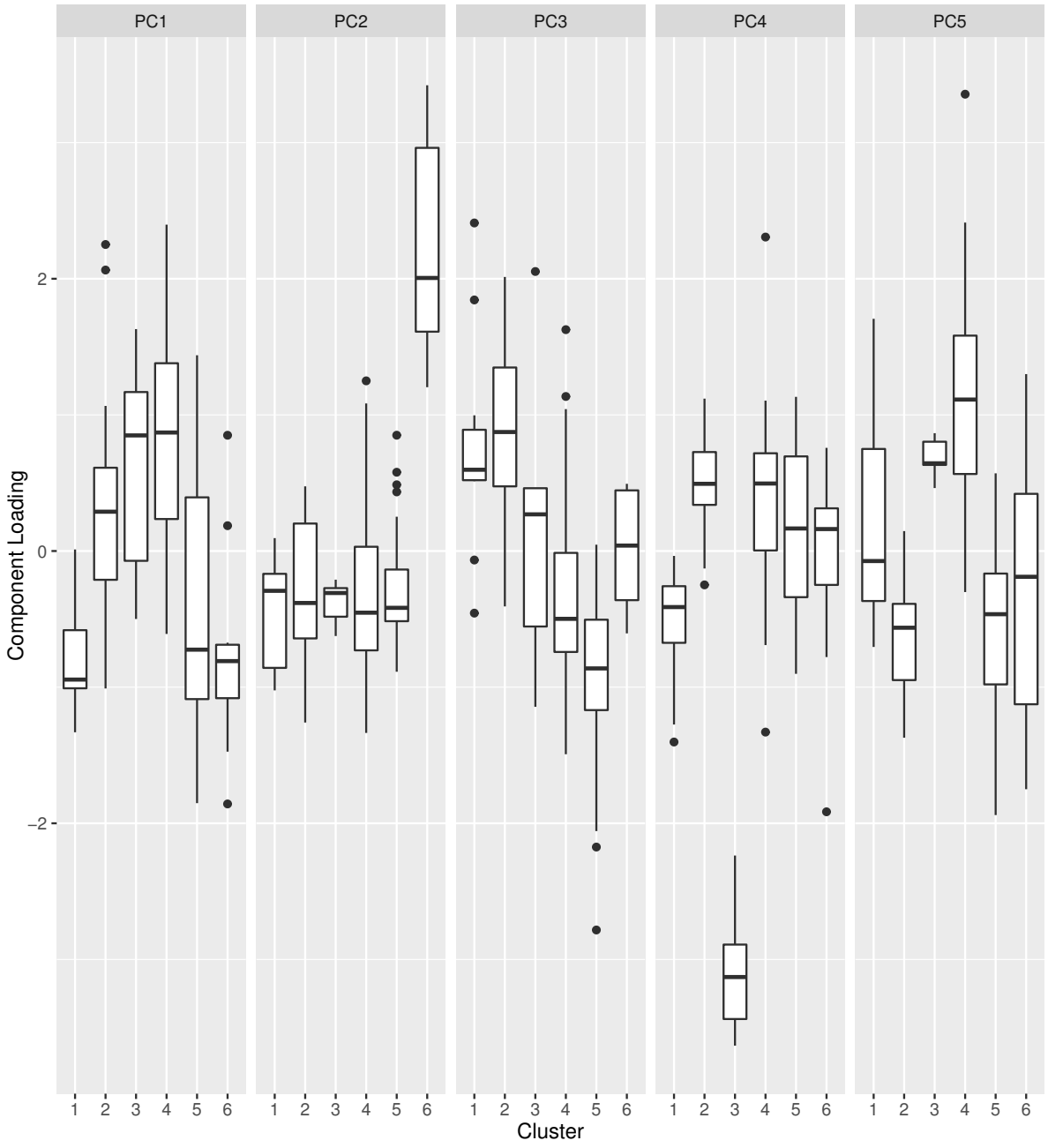


Figure 3.3: Boxplot of individual PCA scores of the 6 management clusters on the 5 PC from table 3.15

Cluster	Atlantis Status	NTotal	Field	Greenhouse Control		Laboratory Analysis			
			Infest	Atlantis WG	MaisTer fluessig	EMR	P197X	W574X	N
1	I	1	-0.46 ±	0.67 ±	0.88 ±	0.09 ±	0.1 ±	0.09 ±	1
1	R	2	0.17 ± 0.27	0.4 ± 0.11	0.74 ± 0.15	0.27 ± 0.03	0.04 ± 0.04	0.11 ± 0.02	2
1	S	8	-0.18 ± 0.15	0.78 ± 0.07	0.87 ± 0.06	0.02 ± 0.01	0 ± 0	0.01 ± 0.01	8
2	I	3	1.01 ± 0.81	0.75 ± 0.16	0.8 ± 0.2	0.16 ± 0.06	0.22 ± 0.15	0.26 ± 0.21	3
2	R	3	0.67 ± 0.33	0.4 ± 0.08	0.67 ± 0.16	0.31 ± 0.14	0.19 ± 0.07	0.37 ± 0.18	3
2	S	18	-0.18 ± 0.13	0.92 ± 0.03	0.99 ± 0.01	0.02 ± 0.01	0 ± 0	0 ± 0	14
3	I	2	-0.32 ± 0.22	0.66 ± 0.11	0.99 ± 0.01	0.05 ± 0.05	0.17 ± 0.04	0.02 ± 0.02	2
3	R	1	0.58 ±	0.14 ±	0.79 ±	0.07 ±	0.62 ±	0.09 ±	1
3	S	3	-0.23 ± 0.21	0.9 ± 0.04	1 ± 0	0.03 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	3
4	I	7	0.21 ± 0.22	0.81 ± 0.04	0.89 ± 0.03	0.07 ± 0.02	0.05 ± 0.03	0.09 ± 0.04	6
4	R	12	0.43 ± 0.15	0.4 ± 0.08	0.59 ± 0.09	0.24 ± 0.07	0.15 ± 0.05	0.39 ± 0.09	12
4	S	5	-0.27 ± 0.07	0.8 ± 0.07	0.99 ± 0.01	0.05 ± 0.03	0.01 ± 0.01	0.05 ± 0.04	5
5	I	10	-0.3 ± 0.2	0.78 ± 0.04	0.86 ± 0.08	0.14 ± 0.1	0.12 ± 0.06	0.19 ± 0.09	9
5	R	15	0.43 ± 0.19	0.34 ± 0.05	0.52 ± 0.08	0.29 ± 0.05	0.06 ± 0.02	0.49 ± 0.06	13
5	S	3	-0.48 ± 0.12	0.88 ± 0.07	1 ± 0	0 ± 0	0.01 ± 0.01	0 ± 0	2
6	I	2	-0.21 ± 0.19	0.51 ± 0.06	0.84 ± 0.01	0.16 ± 0.12	0 ± 0	0.01 ± 0.01	2
6	S	11	-0.17 ± 0.22	0.93 ± 0.04	0.95 ± 0.03	0.01 ± 0.01	0.01 ± 0	0 ± 0	10

Table 3.16: Clusters with corresponding Atlantis Status, scaled infestation level, observed greenhouse control for Atlantis WG and MaisTer fluessig and frequencies for the different target-site mutations and non-target-site plants found per group

Cluster	1	2	3	4	5	6
1	1.00	1.00	0.76	0.02	0.00	0.37
2		1.00	0.30	0.00	0.00	0.69
3			1.00	0.31	0.06	0.23
4				1.00	0.64	0.00
5					1.00	0.00
6						1.00

Table 3.17: P-Values of Fishers Exact Test for count data for the analysis of occurrences of R, I and S within each 6 clusters identified.

3.4 Discussion

Chapter 2 gave an overview of the sampled fields and resistance patterns found. The question at hand was to identify key factors based on field history information that can explain the differences observed. Field management data and soil attributes served as a basis as they were identified as key features in both the establishment of high population sizes and the selection and accumulation of resistant individuals within a field (section 1.2). Among the factors that influence the development of a resistant population, population size and the mutation rate are of high importance (Jasieniuk et al., 1996). While farmers are only able to influence the population size and the selection pressure through management practices, the mutation rate is considered to be constant and uninfluenced by agronomic practices. It can therefore be concluded that under the assumption of a constant mutation rate and in the absence of a fitness penalty of resistance, factors influencing the population in size and composition explain the differences observed in chapter 2. Chapter 2 identified that resistance development on every field occurs individually distinguishing most of the fields from each other. In the absence of resistance groups that could be identified from Chapter 2, the fields were grouped into a categorical system of R, I and S to describe them. This is sometimes difficult since resistance classification based on categories developed to distinguish the samples derived from observations from different years are not always easily applicable (table 3.7). The classification given here is therefore only an attempt to describe the variously observed resistance patterns in their different developmental stages. This accounts for the discrimination between S and I as much as for the discrimination between I and R. Besides the management by farmers, there are also environmental factors, namely soil characteristics, that result in differences in the population size (Menck, 1968; Kruecken, 1975). Soils influence the population of *A. myosuroides* in several different ways, influencing the population. The first is the absolute carrying capacity, meaning the absolute number of plants that can be sustained by the soil in a given location. Second, different soil classes create different environments that are either more or less suitable for the crop and/or *A. myosuroides* . This means that a difference in soil can alter the yield potential of the weed and/or the crop. Third, soil characteristics influence the tillage quality with e.g. soils in which tillage needs to be done in time for suitable seedbed quality for the crop and good efficacy of soil active herbicides. Optimal timing might not be given in fields with different soil types occurring. Fourth, based on the higher genetic variability of weeds compared to crops the weed might be better adopted to the situation occurring in the field e.g. water logging, providing it with a competitive advantage.

Both the habitat characteristics (Soil) and the filters applied by farmers (Crop management) decide on the final composition of the population (Booth and Swanton, 2002). Tiffin and Ross-Ibarra (2014) stated that the local adaptation may occur from genes that have a selection advantage in one environment but are neutral in others or by alleles that posses some kind of environment-dependent fitness trade off. Both apply to herbicide resistance as they provide a fitness benefit in the event of herbicide application (survival). However some of them are also reported to have a fitness penalty in the absence of herbicide selection (Menchari et al., 2007a). Jasieniuk et al. (2008) therefore suggests that the variation in resistances observed reflects the diversity in management patterns.

3.4.1 Soil characteristics

Soils in general define the basis for both the choice of a management regime and the suitability of a field for a given weed species. How different soils influence the presence of *A. myosuroides* was already documented by Kruecken (1975). In general, the habitat preferences for *A. myosuroides* are towards soils that are high in clay (>25%) and moist (Ellenberg, 1979; Menck, 1968). Kruecken (1975) observed a higher abundance of *A. myosuroides* in fields that showed a higher clay content. Ellenberg (1979) described the preferences of *A. myosuroides* as a plant mostly found on wet and semi-wet fields that are slightly acidic to slightly alkaline and are high in nitrogen. Most of these attributes apply to the fields assigned to cluster 2 (figure 3.1). This cluster is mostly found on fields and areas that were previously or still used as grassland. Another interesting observation is that this cluster occurs in locations where most of the resistance problems are observed (Location Z and Location M, figure 3.2). Multiple fields can lie within different clusters of soil, however, an attempt to relate observational data directly to a soil cluster failed (data not shown). As farmers adopt their management practices to a given location, both have to be assessed together Brückner (1958). Findings, e.g. Balgheim (2009); Cavan et al. (1998), suggest that both sensitive and resistant plants occur in patches within a field. Here, bulk samples for the entire field were collected, precluding any analysis of this factor in greater detail. The applications were mostly carried out on the entire field and so additional information might not result in two different applications on the fileld at present. That said, accurate discrimination between resistant and sensitive patches remaining at harvest can provide an early warning for farmers. Further research on the dynamics of resistant weeds on the field level is therefore necessary. Interviewing farmers, patchy weed distributions were often mentioned in their reports of localized *A. myosuroides* problems in one field in comparison to others. Farmers noted that the soil composition in such

patches was typically heavy with wet spots. Containing an *A. myosuroides* favorable soil like cluster 2, could mean that seed production of *A. myosuroides* is higher in these spots due to a sub-optimal crop stand, as Menck (1968) already described. Among the other clusters identified there were several that partially matched the criterion of being highly preferred by *A. myosuroides* . Elevated water levels were also observed in cluster 1 and partially in clusters 5 and 8, indicated by the high score of these clusters on PC1 (figure 3.1). Among these clusters only cluster 8 shows a low score on PC3, with a high pH, higher clay content and reduced water permeability, making them prone to water logging. However, this soil also rarely occurs in the sampling area, making it difficult to assess its individual input on resistance development. The most prominent soil cluster 6 meanwhile shows lower water levels and clay content than cluster 2. Its directional loading on the different components is very similar to cluster 2, however. Among the soils that are high in clay, clusters 4 and 7 were identified as having lower water levels and a higher water permeability suggesting that water logging is not a problem there. However, knowing these nuances in soil composition can be important as the development of large *A. myosuroides* populations in a given location under favorable management can lead to a high population size that increases the chance of resistance developing. As Brückner (1958) describes there are two main regions with *A. myosuroides* in Germany. One in northern Germany including the Hannover area, areas along the Elbe river and the North Sea and a second part in Baden-Württemberg where this study was conducted. It is therefore important to answer to the question of why *A. myosuroides* is an established weed there, causing problems in this area. Soil characteristics as part of the habitat are one answer to this question. The high reliability in the identification of problematic soil clusters 2 and 8 and their separation from clusters 1 and 6 as the two predominant soils makes a transfer of that knowledge to other areas possible if appropriate data is available (table 3.12).

High population sizes, more likely in cluster 2, increase the chance of having resistance under a fixed mutation rate, as pointed out by Jasieniuk et al. (1996).

3.4.2 Crop Management

Management practices can be divided in non-chemical and chemical measures. Among the non-chemical measures the toolbox ranges from summer crop sowing to ploughing, to delayed seeding and making the crop more competitive by enhancing the crop stand through competitive varieties and dense sowing (Lutman et al., 2013). Recently, seed harvesting has been proposed as another tool to reduce the enrichment of the soil seedbank

with weed seeds (Walsh et al., 2012). While the latter might not be suitable for *A. myosuroides* as most seeds are shed before crop harvest, all the others apply (Moss, 1983). Field history information was therefore assessed and variables that describe both non-chemical and chemical measures defined. A static time frame (of six years) rather than crop rotation was used as the range to calculate variables from. This system was chosen as it better represents the selection pressure on the field over a period as it really happened, rather than giving a general statement of the applied measures. Defining variables using crop rotation created the additional problem that annual variations from the planned rotation were difficult to implement in the assessment. The analysis of the non-chemical measures reveals that in general fields that are still considered sensitive, crop rotation is broader with more summer crops than to resistant fields (table 3.13). **Fields consisting of more than 1/3 of summer crop in the rotation were never found to be resistant**, fields comprising 1/6 of summer crops showed a lower chance of developing resistance. The effect was significant for all three groups assessed.

No difference was observed between the ratio of dicot crops in the rotation, which was mostly oilseed rape (table 2.3). This was lready observed by Beckie et al. (2008) who was not able to link resistance to the number of specialty crops grown. While one crop by itself says little about the diversity in the crop rotation, the authors did find that farmers growing more than three different crops encountered a reduced risk of herbicide resistance development. Table 3.13 suggests this as well as only the comparison of I/R was not significantly different for this factor. While the majority of fields showed three crops, fields with a crop rotation of four or more crops were mostly found to be sensitive. Beckie (2009) found in his survey that high reliance on cereals, with more than four in a six year rotation, increases the chance of resistance. The same ratio was found with fields that had less than four times winter cereals in the six year rotation and are less likely to be grouped into R. This goes hand in hand with the patterns for summer crops described above. Interestingly the amount of corn in the rotation was not too different among the groups assessed and only barely significant for the S/R comparison (p=.04). This does not contradict earlier findings but based on our observations there were only few fields with corn in the rotation.

Ploughing on the other hand was only statistically different between S/R. Table 3.15 indicates that this was more attributed to the farming system. Farmers that apply more Integrated weed management (IWM) tools also ploughed more often while others that were using less IWM had more GrpB_Applications. It was considered surprising that both the number of different molecules and the number of MoA was not statistically different

among the groups assessed. Délye et al. (2010b) found no difference between the number of herbicide applications and resistance considering all MoA applied. Table 3.14 agrees partially with these findings. The total number of herbicide applications is statistically different between the S/R comparison but not between the other comparisons tested. The specific grass weed applications was statistically different between all comparisons besides S/I. In terms of specific ALS-inhibitor applications only I/R were not statistically different. In general it can be concluded that in terms of total herbicide applications, intermediate fields are closer to the sensitive fields. However, in terms of the specific selection pressure by a Group B herbicide against *A. myosuroides* intermediate fields show higher similarity to resistant fields. The unique application pattern at the field level however shapes a unique fingerprint as found in chapter 2 (Délye et al., 2010b). Resistance cannot therefore be viewed in light of herbicides alone but needs an assessment of the entire cropping system over a period of time that is long enough to assess all factors shaping the selection of resistance properly (e.g. the six years studied here). This means that the speed of resistance development and therefore the status observed today is determined by both chemical and non-chemical measures. The use of a wider range of MoA was not found to be a significant factor in discriminating the resistance status.

As indicated by table 3.15 corn means a very adapted system that is applied more frequently by farmers with cattle or biogas production than those farmers having pigs and mostly grow winter cereals. The typical corn rotation observed contains a rotation of winter wheat and corn and results in reduced usage of different MoA (figure 3.3). After corn late seeding is typically observed for the subsequent crop which reduces the option of an pre-emergent compound, mainly flufenacet, in autumn. This explains both the reduced variability in herbicides and the reduced number of herbicide applications. Late seeding by itself is seen to discriminate well between resistant fields that rarely practice it and S/I on the other hand where it is applied in both groups on some occasions. Glyphosate treatment is often observed to spray the cover crop before seeding in the corn crop rotation. ACCase-inhibitors are seldom seen in a crop rotations mostly comprising corn and winter wheat, resulting in high ALS-inhibitor usage as indicated by a lower score on PC3 (table 3.3) which distinguishes them from farmers in cluster 1 and and 2 who are using a wider toolbox of integrated weed management tools. The group of corn farmers does not contain any resistance cases so far (table 3.16). Among the other groups identified there were two which practice IWM and those who rely less on IWM. Among those who do (cluster 1 and 2) a higher herbicide use intensity is observed in cluster 2. This cluster also shows the highest use intensity of integrated weed management tools like summer crop

use and ploughing. Both result in the majority of fields in these two clusters remaining sensitive. Clusters 3 and 4 showed an even higher treatment frequency while reduced use of IWM with fewer fields being classified as resistant in cluster 3 than in cluster 4. A very strong difference was observed in the soil composition of these two clusters. Fields in cluster 3 comprised both shallower but heavier soils with an often reduced frequency of ploughing and wet soils present. Cluster 4 shows a very strong value on PC5, implying that fields in this cluster are higher and wetter than those in other clusters. The heavy fields that are associated with grassland are often sown early with a winter crop as moisture in autumn can permit field work. The moisture also prevents the early accessibility of the fields in spring, preventing the preparation of an appropriate seed bed and earlier sowing dates that are sought for high yields of summer crops. Farmers therefore tend to grow early sown winter crops on those fields that favor *A. myosuroides* greatly(Kemmer et al., 1980). The often reduced tillage that quite frequently occurs supports this further. In light of suitability for *A. myosuroides* that would mean that a lack of diversity in management together with very favorable conditions for *A. myosuroides* explains the different abundances of resistance status between these two clusters. These differences in occurrence of R, I and S were not statistically different, however (α=.05, table 3.17), probably due to the low sample number in cluster 3. Significant differences were found between clusters 3 and 5. Compared to cluster 3, cluster 5 showed a lower diversity and a lower frequency of herbicide applications though the cropping regime was dominated by winter cereals. Soils were not amongst those being considered favorable for *A. myosuroides* . As the frequency of resistant fields was higher in cluster 5 than in cluster 3 one can say that the little addition of IWM in cluster 3 made this soil more robust even under more *A. myosuroides* favorable conditions than the low diversity found in cluster 5. As stated earlier, the different patterns in terms of target site mutations and greenhouse efficacy of the various products was not distinguishable between clusters and therefore could not be linked to a given management regime. More field history samples might be necessary to explain the differences observed, however, as stated in chapter 2 every field seems to be unique in terms of its resistance development pattern. This would indicate that due to target-site mutations selected from the standing genetic variation, the final composition of resistance was predetermined prior to the application of resistance, otherwise a specific resistance pattern would have been attributed to a specific herbicide pattern. While this accounts for TSR the situation is different for EMR which can show linked resistances to several MoA. Here, a preselection of several candidate genes must have occurred prior to the introduction of ALS-inhibitors to the market as it was presently found by others

(Knight, 2016). For *A. myosuroides* the most likely selection was by ACCase-inhibitors , namely FOP/DEN as DIM control EMR plants (chapter 2). Therefore, the final composition of resistance in the field is only partly influenced by a herbicide (selection pressure) that selects spontaneously occurring genes from a predetermined population. However, it could be shown that 1) different management practices show different risks of resistance development and 2) similar management regimes might yield different resistance statuses under different soils.

Besides the management data provided by the farmer there are also several soft factors, like awareness of resistance development, the accuracy and quality of the measures applied and the degree of training in agronomy, that are reported to influence resistance development (Beckie, 2009; Hogrefe, 2011). There were no attempts to assess these factors in the scope of this study however, though differences among the farmers in the perception of the problem were observed while carrying out the interviews. No assessment of crop competition was possible as most farmers were only able to provide information on varieties and seeding densities for the most recent years but did not have records apart from these. Herbicide use rates as a prominent example to influence metabolic resistance in particular (Neve and Powles, 2005b) was also not assessed because the herbicides were always sprayed with the recommended label use rate. That does not suggest that situations occurred which might be considered to use sub lethal dose rates, in particular with late applications to well developed ALOMY plants. This phenomenon can be explained by the high level of animal husbandry practiced among the farms in this area. On farms where animal husbandry has priority, less time remains for arable work resulting in full doses being applied to tackle the problem right away. Beckie (2009) reported following a survey that bigger farms are more prone to resistance development as they are under more time pressure. This is a valid conclusion but has to be generalized both according to farm size and to the labor available to apply measures at the right time. These findings show the general problem of treating weeds with label use rates at sub-optimal time points e.g. later growth stages of the weeds. The size of the farm might only have an indirect effect as the chances of finding resistant individuals per farm increases with its size. The effect therefore of application timing was not assessed here as it would require knowledge of the weed growth stage at time of application. Approximating this from the crop, the seeding date and time of application is rather inaccurate and was therefore not done. *A. myosuroides* developmental stages follow the crop in which they occur, resulting in a difference in the correct timing from field to field. This highlights the importance of studying the interplay of both soil and management. However, as stated before, man-

agement is not independent of soils and a farmer will choose an adopted crop rotation that suits the needs of their farm according to criteria beyond weed control. Therefore an understanding of the risk that occurs at the individual field level is key in consulting farmers with appropriate management tools. Especially since clusters 4 and 5 from table 3.16 are the biggest ones in terms of fields that are comprised within. Bagavathiannan and Norsworthy (2014) and Beckie (2009) suggest that early stages of resistance detection are the most critical, though they point out that this is the hardest point for farmers to identify. While these low (but significant) frequencies are also difficult to detect in the greenhouse and laboratory, the following chapter will focus on the prediction of resistance at the field level.

Chapter 4

Predicting Herbicide Resistance: A Comparison of Different Methods to Forecast an Event of Resistance to ALS-Inhibitors in *Alopecurus myosuroides* Huds.

4.1 Introduction

Prediction of herbicide resistance has long been in the focus of herbicide resistance research (Richter et al., 2002; Colbach et al., 2006; Rummland et al., 2014; Neve et al., 2003, 2011; Renton et al., 2014; von Redwitz et al., 2016). These approaches focus all on simulation models which resemble the life cycle of a weed over different years. A brief summary on the development of such a model and the aspects to consider is given by Holst et al. (2007). Parameters describing relevant biological processes and their exact quantification is therefore crucial for an accurate simulation (Renton et al., 2014). While this parameterization is mostly done using field trials and/or small experiments, the validation of such tools is more complicated. The availability of datasets, ideally from multiple years of independent test cases, are necessary for true validation. Holst et al. (2007) points out that this point is often not considered however. Furthermore, different focuses exist for the development of a simulation model. While some are developed for decision support, others were developed to gain insights into system dynamics or the influence of one or multiple parameters on the entire system. The advantages of modeling is that it allows large scale virtual experiments to be carried out without the need for extensive and lengthy field experiments as well as its fairly inexpensive set up compared with these experiments. Numerous models for different weeds and purposes have been developed so far and are summarized in reviews by Renton et al. (2014) and Holst et al. (2007). One model widely applied is the RIM (Ryegrass integrated management) model developed for *Lolium rigidum* in Australia (Lacoste and Pow, 2015). The model is used by farmers in Australia to predict the development of the weed and its effect on their yields. Broad application was achieved by having an easy to use interface together with farmer relevant results. Different management scenarios can be tested and compared to each other. The simulation also accounts for herbicide resistance by allowing the user to reduce the efficacy of a given herbicide. No genetic simulation is applied. The purpose is to compare different strategies to choose sustainable management systems for *L.rigidum*.

Many simulations compare different scenarios on a relative basis. This means that based on multiple simulations of one dataset with random parameters, the risk of resistance is defined based on the number of times the simulation discovers resistance. Rummland (2015) for *Apera spica-venti* and Knight (2016) for *A. myosuroides* attempted to accurately validate their models with data obtained in the field. The approach by Rummland (2015) was able to describe the resistance situation in the field accurately well and could be used to determine the resistance risk on the field level. However, to apply this approach knowledge of the soil seedbank is essential, something which is difficult and

time consuming to obtain. The approach by Knight (2016) also follows the development of resistance to *A. myosuroides* with the inclusion of EMR as a monogenic trait in the simulation. However, the simulation did not yield the desired accuracy by the author.

The focus here was thus on providing a tool set capable of predicting the resistance situation and individual field risk accurately by using parameters that can be easily obtained by farmers. Farmers should be able to use such a tool on their own fields and draw valid conclusions from the results. Results found in the previous chapter allow the development of such a tool. Points that were considered based on these earlier findings are:

- Resistance develops individually in each field and the influx of resistance genes/alleles is small to marginal (chapter 2).
- Resistance is more likely to occur on soils that are favorable for high *A. myosuroides* populations (chapter 3).
- Crop management including the herbicide regime practiced by the farmer is therefore the decisive factor for the development of resistance (chapter 3).

Multiple variables related to soil and management were considered in the development of such a tool. Two different approaches were used to predict the resistance development and describe the situation in the field.

One was a supervised learning technique (Random Forest) and the other a simulation model. The choice for the two different approaches was made following analysis of the specific strengths and drawbacks of each of them, as discussed below.

Supervised learning techniques such as random forest are tools that train an algorithm based on a training data set with defined outcomes (e.g. R/S). This algorithm is then applied to a test data set. Since random forest applies multiple decision trees to the same randomly chosen data subset, its accuracy is high (Breiman, 2001). However, accuracy of these kinds of algorithm generally increases with the training data size assuming that all relevant features (variables) are considered in building the algorithm (Banko and Brill, 2001). Since they require real life scenarios to be established, their set up for the purpose of resistance definition is less difficult compared with a simulation model as discussed below. Chapter 3 identified key management groups which differentiate resistant from sensitive cases reasonably well. Providing management data in the training of the algorithm in addition to the soil characteristics should enable the establishment of a robust algorithm. The drawback of this approach is the provision of sufficiently large and representative data as this can be difficult to obtain from farmers. Furthermore,

with smaller sample sizes outliers or samples that are not representative can lead to a misclassification using the algorithm (Brodley and Friedl, 1999). In contrast to simulation models, these algorithms work like a black box and do not let the user draw conclusions about the underlying relationships leading to the output. This might be one reason why they are not yet very common in weed science. Another problem is the constant need for readjustment of the algorithm as the selection by the farmer evolves. Since a resistance status reflects the situation at one specific point, a possible change in the status in five years would require a readjustment of the algorithm. Further problems can arise from the selection of samples used to train the algorithm. Crop rotations that follow different principles than the ones provided in the training dataset might be classified incorrectly if the algorithm is not able to generalize sufficiently. A broad training data set is thus necessary, leading again to the previously stated problem of getting a sufficiently large sample size.

Simulation models meanwhile account for the problem of readjustment as a correct adjustment of the relevant parameters permit an extrapolation beyond the present. However, the correct assessment of all parameters is difficult especially as some might differ significantly between fields (e.g. seedbank size, Moss (1985)) or years (e.g. germination rate, Zwerger (1993)). Attempts by Knight (2016) show the difficulties in establishing a working simulation model that is both scientifically sound and validated. Implementing ideas on both the genetic differences observed would require a simulation that is able to consider differences in soil, management and resistance genes. Such ideas have been implemented separately by several authors (Cousens and Moss, 1990; Zwerger, 1993; Dunker et al., 2002; Richter et al., 2002; Böttcher, 2003; Neve et al., 2003; Colbach et al., 2006; Bagavathiannan and Norsworthy, 2014; Rummland et al., 2014; Knight, 2016). Combining these elements and implementing the findings of previous chapters will therefore be the objective for this chapter. New features of the simulation combine 1) soil properties (Dunker et al., 2002), 2) the consideration of flushes at different cohorts and at different layers (Cousens and Moss, 1990; Neve et al., 2003; Knight, 2016), 3) the implementation of a complex genetic model including more than one locus (Böttcher, 2003; Bagavathiannan and Norsworthy, 2014; Knight, 2016) into one model. Parameterization will be performed using literature data, findings of previous chapter and experimentation. This work attempts to establish a model that accurately predicts the outcome of a given selection by the farmer by alternating several variable combinations.

As shown in previous chapters, observations of resistance can be linked to certain soil and management data. As systems were found which have a higher occurrence of R

than others, it is believed that these differences are not due to random chance but the underlying selection factors described earlier. It is important here to break down the complexity of what is observed to allow only relevant patterns to be provided to farmers. The outcome of either tool is evaluated based on the correct classification of a case based on the R, I and S rating scheme presented earlier.

The work should develop an applicable tool for use by crop advisers and farmers. For the simulation model in particular, the requested input needs to be restricted to the information they can provide. Computation speeds need furthermore to be at reasonable levels to allow quick comparisons between different management systems. The aim is to provide a valuable tool to successfully prevent or delay resistance development. The assessment of the two approaches to achieve this and the combination of them that is ideally achieved, will lead to high accuracy.

4.2 Material & Methods

The resistance status of many *A. myosuroides* populations has been described in chapter 2. An analysis of the resistance evolution and its causes was done in chapter 3. Here two approaches that should allow the prediction of the resistance status of individual fields are presented. One approach uses a supervised learning technique (Random Forest) while another uses a population simulation model. Both were used to assess their suitability for the topic. While supervised learning techniques have not yet been established for that purpose, several population models for resistance prediction in different weeds have been developed e.g. Neve et al. (2003); Colbach et al. (2006); Rummland et al. (2014).

4.2.1 Supervised Learning

The random forest approach was applied using the randomForest package in R (Liaw and Wiener, 2002). Herbicide resistance data from the 106 fields in the previous chapter with their corresponding field history indices and soil characteristics were used in the analysis. The data were randomly split into a training and a test dataset with 70 and 36 samples respectively. For easier interpretation of the results the predefined Atlantis status was further simplified to R and S only. All fields are characterized as I being counted as R. The algorithm was trained using 1500 random trees generated from a random sample of 25 fields without replacement. The final node size option for the tree was set to 4 to account for potential over-fitting of the algorithm. The frequency of classifications for R and S were calculated for the test dataset and compared to the defined Atlantis status . A new variable (SV_UW) was introduced in the analysis as a measure for diversity of *A. myosuroides* management each year. The variable describes the number of different measures (delayed seeding, ploughing, summer crops and the use of multiple MoA that was done by the farmer). Each measure applied within a year gets a score of 1. SV_UW ranges therefore between 0 and 4. E.g. in winter wheat, using two modes of action and ploughing thus results in a score of 2 while shallow tillage and only one mode of action would result in 0. The values are averaged for the time frame of the 6yrs under consideration.

4.2.2 Resistance Simulation Model

A population model was developed following the example by Richter et al. (2002) and Böttcher (2003). It attempts to present the entire life cycle of a *A. myosuroides* population over multiple generations under different weed management scenarios. The model

uses a cellular automaton (CA) as its basis to simulate the resistance development within a field. A genetic model using three loci was established as an extension of the work of Böttcher (2003). Spatial spread within the field was simulated by modeling interaction of the cells of the Cellular Automaton (CA). During the year the plant develops through different phases that are modeled here and presented in the layout of the model shown in figure 4.1.

The model consists of two main phases. First part is the initialization phase in which parameters for the field are determined. This phase consists of two steps:

1. Determination of the soil characteristics and the resulting soil properties
2. Determination of the initial soil seedbank

The second part is the simulation phase running in loops with every year comprising one loop. The critical phases in the season that are simulated for the population are:

3. The soil seedbank phase under the influence of age dependent decay and tillage
4. The germination phase
5. The developmental phase (influenced by herbicides)
6. The production of seeds

Cellular Automaton (CA)

The CA consists of 10 by 10 cells with $C_{x,y}$ referring to a specific cell. Every cell represents 100sqm. This size was chosen to allow reasonable computation times in order to test many combinations of the model. The model shows the development of an *A. myosuroides* population over a given time period to predict the development of this population under the influence of different management regimes, and their impact on resistance to multiple modes of action. Three distinct and independent loci (target site mutations) representing resistance to ALS-inhibitors (Locus A with AA, Aa, aa), ACCase-FOP/DEN (Locus B with BB, Bb, bb) and ACCase-DIM Locus (C with CC, Cc, cc) are modeled in this study. This was chosen to represent the findings of chapter 2. There it was found that for the ALS-inhibitors two different target site mutations occur, with each typically at a separate geographical location (H, M, Z) and hardly together. Therefore ALS resistance was modeled by one locus (LociA). For the ACCase-inhibitors it was found that there are two patterns. Resistance to FOP/DEN herbicides and resistance to DIM herbicides. Both can occur together. Therefore LociB and LociC account for these findings. The

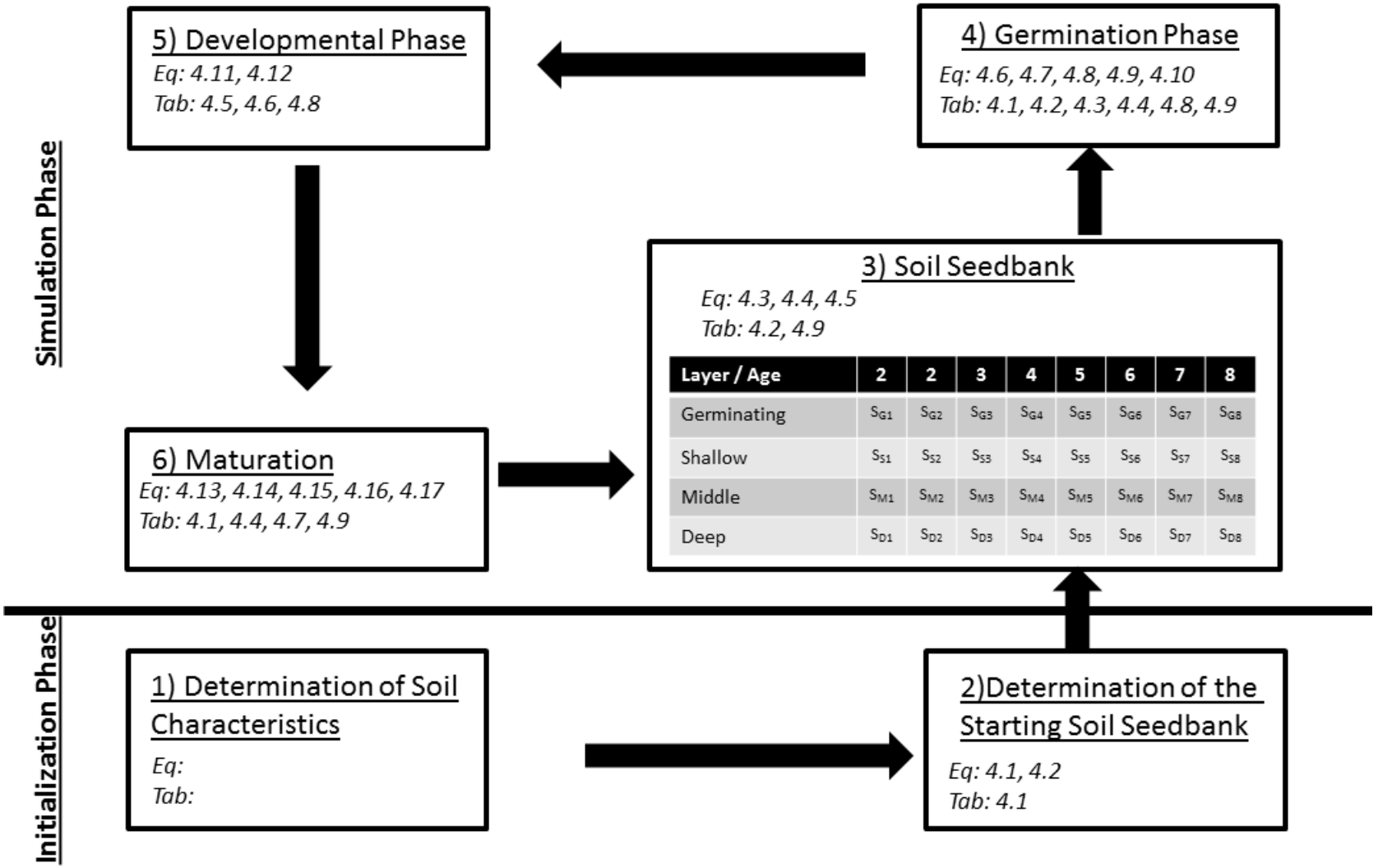


Figure 4.1: Model Scheme with corresponding equation (Eq.) and tables (Tab.) used at each point

survival of an allele following a given herbicide application is presented in table 4.5. A total of 27 different genotypes were formed.

Initialization of the model

1. Determining the soil characteristics and the resulting soil properties

Soil properties as assessed in chapter 3 were used as a basis to develop a CA accounting for different soil classes. Different soil clusters were connected with different biological parameters (table 4.1). Values for seed carrying capacity per sqm of soil (Seeds), maximum number of seeds of *A. myosuroides* per plant in the absence of herbicide application and competition (A_{max}) and maximum number of *A. myosuroides* plants per sqm (D_{max}) were varied between the clusters assessed. The percentage of coverage of every cluster across all fields was taken and adjusted to the number of cells in the CA. For field Z038 21% coverage with cluster 2 and 79% coverage with cluster 4 was found. This results in 21 and 79 of the cells in the CA using the corresponding values from table 4.1 respectively (figure 4.2).

Soil Cluster	Seeds [1/sqm]	A_{max}	D_{max}
1	20000	1	1.5
2	50000	1.25	2
3	10000	1	1
4	10000	1	1
5	20000	1	1.5
6	10000	1	1
7	10000	1	1
8	20000	1	1.5

Table 4.1: Model Parameter settings for different soil clusters derived from section 3.3.1

2. Determining the initial soil seedbank

The number of seeds per cell (TotalSeeds_{xy}) was taken from table 4.1 according to defined soil characteristics from step 1. The number of seeds was varied stochastically between 70% and 130% of the value from table 4.1 to simulate an uneven distribution. Another stochastic component was added by using a binomial function with a success rate of 0.7 to decide whether a cell should contain seeds at all or not. This was done to assure that the distribution of seeds was somehow arbitrary within the field and not uniform among the cells in the CA. The number of seeds contained within all cells of the CA was taken as a basis to calculate the genotype composition. The initial allele frequency of the different loci was set by the Initial Frequency of A in the population (ResistanceFreqA),

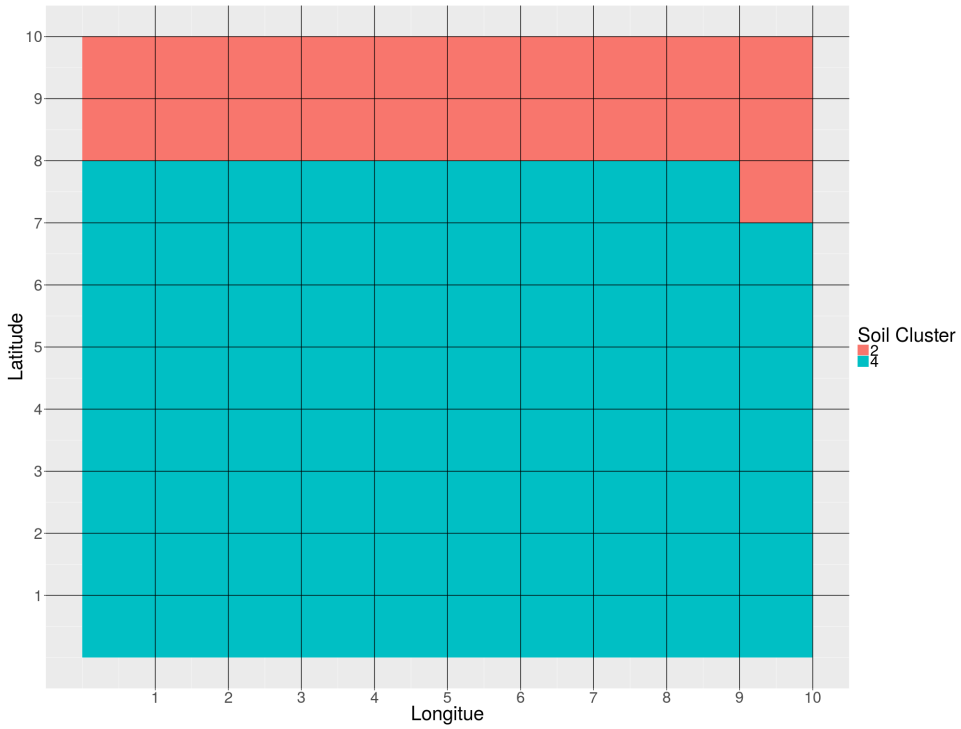


Figure 4.2: Representation of Soil Clusters for Z038 among the cells of the CA

Initial Frequency of B in the population (ResistanceFreqB) and Initial Frequency of C in the population (ResistanceFreqC) for loci A, B and C respectively. The values chosen for the different parameters along with the justification are shown in table 4.9. These frequencies were used to calculate the allele frequencies at each locus according to Hardy-Weinberg. Frequencies of the 27 genotypes were calculated by multiplying the individual allele frequencies at the different loci with each other (4.1).

$$Freq_{LocA,LocB,LocC} = Freq_{LocA} * Freq_{LocB} * Freq_{LocC} \tag{4.1}$$

The frequency of every genotype was taken and multiplied by the total amount of seeds to determine the number of seeds in the soil seedbank carrying this genotype (4.2)

$$Seeds_{LocA,LocB,LocC} = Seeds_{Total} * Freq_{LocA,LocB,LocC} \tag{4.2}$$

These seeds were than randomly distributed among the cells and soil layers according to the total number of seeds present in every cell.

Simulation Phase

3. Soil Seedbank

The seedbank contains all seeds that are produced or were previously added to the seedbank in the initialization phase. The seedbank was divided into different layers of soil according to Cousens and Moss (1990). The four layers are as following: germination layer, shallow soil layer, middle soil layer and deep soil layer. Only seeds in the germination layer representing the first 5cm of soil are considered for germination in the next season. Fatal germination[1] is not considered in the model. Furthermore every soil layer contains seeds from different age cohorts with S_{la} representing the number of seeds in a different layer (l) from a given time cohort (a). It is also assumed that seeds that do not germinate are following a decay in the soil (Seedbank) denoted by the surival rate of seeds in the soil (pS)(eq. (4.3), table 4.9). AgeMatrix is a Leslie matrix with the portion of seeds surviving a year in the soil as a probability of entering the next age layer (4.4).

$$S_{t+1,l} = Seedbank_{t,l} * AgeMatrix' \tag{4.3}$$

$$AgeMatrix = \begin{pmatrix} 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ pS & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & pS & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & pS & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & pS & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & pS & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & pS & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & pS & pS \end{pmatrix} \tag{4.4}$$

The effect of tillage is simulated by multiplying the seeds in every soil layer with a Leslie matrix containing the probability of seeds in one layer being transferred to another (4.5). Matrices for plough, cultivator and shallow tillage were taken from Cousens and Moss (1990).

$$Seedbank_{t+1,a} = Seedbankge_{t,a} * TillageMatrix \tag{4.5}$$

4. Germination Phase

A given fraction (paG) of seeds being in the germination layer are considered for germination. The number of successfully germinating seeds is subtracted from the soil seedbank

[1]Seeds that were primed for germination by tillage but later buried in deeper layers of the soil

(4.6).

$$Seedbank_{t+1} = Seedbank_t * (1 - paG) \tag{4.6}$$

Six cohorts (C_0-C_5, table 4.2) were formed based on values reported by (Kemmer et al., 1980) and own experimentation which represent different seasonal events. The cohorts present critical growth events throughout the season and do not represent actual dates or fixed time points. The success rate of germination is based on different germination time points throughout the season as Kemmer et al. (1980) reports. Values related to the six cohorts were adopted empirically based on the comparison of the modeling result with observed data. The success rate represents the number of plants among those germinated that successfully developed into mature plants. The number of seedlings germinating within a cohort is therefore corrected by the success rate (4.7).

$$Seedlings_{C_x} = paG * GerminatedSeeds_{C_x} * SuccessRate_{C_x} \tag{4.7}$$

Cohort	Time Frame	Targeted Event
C_0	After harvest to mid August	
C_1	Mid August to mid September	oilseed rape seeding
C_2	Mid September to mid October	barley and early wheat seeding
C_3	Mid October to vegetation stopping	late wheat seeding
C_4	vegetation Beginning to Mid March	spring application
C_5	After Mid of march	sugar beet and corn planting

Table 4.2: Description of the six germination cohorts for *A. myosuroides* . Time Frame indicates a rough time within the growing season rather than an accurate time point. The targeted event to be represented by the cohort is indicated.

The established seedlings of every genotype that germinate within a cohort undergo a density dependent function to prevent an unreasonably large number of plants (4.8). The equation was taken from Richter et al. (2000) and adopted to the situation occurring at every cohort. $Dmax_{Genotype,Cx}$ describes the D_{max} value of every genotype at a given cohort and is derived from (4.9). L is the form parameter of the equation and is set to $L = Dmax_{Genotype,Cx}$.

$$Seedlings_{Genotype,Cx} = Dmax_{Genotype,Cx} * \frac{Seedlings_{Genotype,Cx}}{L + Seedlings_{Genotype,Cx}} \tag{4.8}$$

D_{max} values per genotype and cohort ($Dmax_{Genotype,Cx}$) were calculated as in (4.9). $Dmax_{Cx}$ represents the D_{max} value for the given cohort as calculated from (4.10) and is multiplied by the portion of germinated seeds carrying a specific genotype. $Dmax_{Genotype,Cx}$

Crop	Seeding Date	Fraction of Germination [%]						Success of Germination [%]					
		C_0	C_1	C_2	C_3	C_4	C_5	C_0	C_1	C_2	C_3	C_4	C_5
AVESA	normal	20	20	20	20	15	5	1	1	1	1	100	100
BEAVU	normal	20	20	20	20	15	5	0	1	1	1	1	100
BRSNS	normal	20	20	20	20	15	5	1	1	1	1	100	100
BRSNW	late	20	20	20	20	15	5	1	50	100	100	5	1
BRSNW	normal	20	20	20	20	15	5	1	100	100	100	5	1
HORVS	normal	20	20	20	20	15	5	1	1	1	1	100	100
HORVW	late	20	20	20	20	15	5	1	1	30	100	20	0
HORVW	normal	20	20	20	20	15	5	1	1	100	100	20	0
PIBSA	normal	20	20	20	20	15	5	1	1	1	1	100	100
TRZAS	normal	20	20	20	20	15	5	1	1	1	1	100	100
TRZAW	late	20	20	20	20	15	5	1	1	1	100	20	0
TRZAW	normal	20	20	20	20	15	5	1	1	100	100	20	0
TTLWI	late	20	20	20	20	15	5	1	1	100	100	20	0
TTLWI	normal	20	20	20	20	15	5	1	1	100	100	20	0
ZEAMX	normal	20	20	20	20	15	5	1	1	1	1	1	100

Table 4.3: Fraction of germinated Seeds [%] in every cohort and the success of developing to mature plants in the given crop [%]

values of <1 were rounded to 1.

$$Dmax_{Genotype,Cx} = Dmax_{Cx} * \frac{\sum Seedlings_{Genotype}}{\sum Seedlings_{Total}} \tag{4.9}$$

Cohort formation is based on a feedback function where $Dmax_{Cx}$ depends on the number of seedlings (Seeds) of the previous cohorts. $Dmax_{Total}$ stands for the D_{max} value for a given crop as presented in table 4.4. Dunker et al. (2002) uses a general D_{max} value of 500. Here different values for every crop were used based on the value presented by Dunker et al. (2002) and ideas which were adopted based on own experience from earlier chapters.

$$Dmax_{Cx} = Dmax_{Total} - \sum_{C_0}^{C_{X-1}} Seeds_{Cx} \tag{4.10}$$

Equation 4.10 therefore implies that the number of seedlings successfully establishing in spring in particular depends on the number of seedlings that were successfully established in autumn. This means that the simulated number of plants that can successfully establish in spring is lower if there are already high numbers of *A. myosuroides* from autumn. On the other hand, a successful control of fall germinated *A. myosuroides* results in a higher number of successfully established seedlings in spring with regard to equation 4.7. The total number of seeds germinating (paG) is not affected by this feedback func-

tion, since seeds that do not establish successfully have still germinated and therefore decrease the soil seedbank.

Crop	Dmax	FV
Avena sativa L. (AVESA)	250	800
Beta vulgaris L. (BEAVU)	250	50
Brassica napus L. (summer) (BRSNS)	250	50
Brassica napus L. (winter) (BRSNW)	250	50
Hordeum vulgare L. (summer) (HORVS)	250	800
Hordeum vulgare L. (winter) (HORVW)	500	500
Pisum sativum L. (PIBSA)	750	50
Triticum aestivum L. (summer) (TRZAS)	250	500
Triticum aestivum L. (winter) (TRZAW)	500	350
Triticale (*Triticum aestivum* L. x *Secale cereale* L.) (TTLWI)	500	350
Zea mays L. (ZEAMX)	250	10

Table 4.4: D_{max} and FV values per Crop as used in the simulation

5. Developmental Phase

The developmental phase is mainly characterized by the herbicide regime applied by the farmer. Every locus is rated based on the survival when a given herbicide is used (table 4.5). It was assumed that survival of the wild type alleles (aa, bb, cc), heterozygous alleles (Aa, Bb, Cc) and homozygous alleles (AA, BB, CC) is 1%, 90% 100% respectively.

Loci	Flufenacet	PRE (Other)	FOP (F)	FOP (S)	DIM (F)	DIM (S)	ALS (F)	ALS (S)	Alt (F)	Alt (S)	Glyphosate
aa	0.20	0.50	0.01	0.01	0.01	0.01	0.01	0.01	0.20	0.20	0.01
Aa	0.20	0.50	0.01	0.01	0.01	0.01	0.90	0.90	0.20	0.20	0.01
AA	0.20	0.50	0.01	0.01	0.01	0.01	1.00	1.00	0.20	0.20	0.01
bb	0.20	0.50	0.01	0.01	0.01	0.01	0.01	0.01	0.20	0.20	0.01
Bb	0.20	0.50	0.90	0.90	0.01	0.01	0.01	0.01	0.20	0.20	0.01
BB	0.20	0.50	1.00	1.00	0.01	0.01	0.01	0.01	0.20	0.20	0.01
cc	0.20	0.50	0.01	0.01	0.01	0.01	0.01	0.01	0.20	0.20	0.01
Cc	0.20	0.50	0.90	0.90	0.90	0.90	0.01	0.01	0.20	0.20	0.01
CC	0.20	0.50	1.00	1.00	1.00	1.00	0.01	0.01	0.20	0.20	0.01

Table 4.5: Alleles and the rate of survival when a given herbicide is applied

The effectiveness of the applied herbicides is related to the cohort it is covering and the amount of seedlings present. The effectiveness of a given application in a given cohort

can be seen in table 4.6. As an example, a treatment with Flufenacet affects all seedlings that successfully established in the cohort C0-C2 but not C3-C5.

Cohort	Flufenacet	PRE (Other)	FOP (F)	FOP (S)	DIM (F)	DIM (S)	ALS (F)	ALS (S)	Alt (F)	Alt (S)	Glyphosate
C0	1	1	1	1	1	1	1	1	1	1	1
C1	1	1	1	1	1	1	1	1	1	1	0
C2	1	0	1	1	1	1	1	1	1	1	0
C3	0	0	1	1	1	1	1	1	1	1	0
C4	0	0	0	1	0	1	0	1	0	1	0
C5	0	0	0	0	0	0	0	0	0	0	0

Table 4.6: Cohorts and herbicide applications that cover them, where 1 denotes yes and 0 denotes no

The number of plants surviving (JP) both treatments is derived as in (4.11) with the survival rate of the autumn application S_{AppHB} and the survival of the spring application S_{AppHB}. Both values were set to 1 where either application was not carried out. If two applications occur together, the survival rate is derived by forming the product of the two individual herbicide survival rates for a given genotype. An example would be the application of Alt (S) and FOP (S) together in spring in a winter barley crop. The survival of the genotype aabbcc is 0.01 and 0.2 for FOP (S) and Alt (S) respectively (table 4.5). Therefore the survival of this genotype would be $0.002^{*}Konst_{Spring}$ (table 4.5, table 4.8). For the genotype aaBBcc the survival is 1 and 0.2 for FOP (S) and Alt (S) respectively, resulting in a total survival of $0.2^{*}Konst_{Spring}$ for this genotype.

$$JP_{Genotype} = \sum Seedlings_{Genotype,Cx} * S_{AppHB} * S_{AppFr} \tag{4.11}$$

The total number of plants at the end of the season (MatPfl) is the rounded down value of JP to the next integer (4.12).

$$MatPfl = \lfloor JP \rfloor \tag{4.12}$$

6. Maturation

The number of seeds per plant (number of seeds produced per plant in a given cell

(SeedPlant)) is calculated by Eq. (4.13) (Dunker et al., 2002).

$$SeedPlant_{Genotype} = MatPfl_{Genotype} \frac{A_{max}}{(1 + Form_A * MatPfl + Form_B * FV)^{Form_{Beta}}} \tag{4.13}$$

$Form_A$, $Form_B$ and $Form_{Beta}$ are constant with values of 0.3, 0.3 and 0.5 experimentally determined and adopted from Dunker et al. (2002). A_{max} represents the maximum number of seeds produced under perfect conditions in the absence of competition. FV describes the crop density per sqm as derived from table 4.4.

The number of haplotypes ($SeedHP_{haplotype}$) in the newly formed seeds is derived by multiplying $SeedPlant_{Genotype}$ with the corresponding value for haplotype formation (HP_{Form}) of table 4.7 for every genotype (4.14).

$$SeedHP_{Haplotype} = SeedPlant_{Genotype} * HP_{Form} \tag{4.14}$$

The sum of every haplotype formed from each genotype per cell is the total amount of seeds with this haplotype ($SeedHP_{haplotype}$). The frequency of every haplotype in the pollen cloud is derived from the share of every haplotype among all haplotypes formed (4.15). Equations 4.14 and 4.15 were adopted from Böttcher (2003).

$$Pollen_{haplotype} = \frac{SeedHP_{haplotype}}{\sum SeedHP_{Total}} \tag{4.15}$$

The final number of new seeds ($SeedsNew$) formed is calculated by forming the outer product (Kronecker Product) of $SeedHP_{haplotype}$ and $Pollen_{haplotype}$ (4.16).

$$SeedsNew = SeedHP_{Haplotype} \otimes Pollen_{haplotype} \tag{4.16}$$

A mutation rate is introduced to simulate the formation of newly resistant seeds under a given mutation Mutation Rate (MutRate). The newly mutated seeds (MutSeeds) are calculated by Eq. (4.17). The mutation rate was assumed to be the same for all three loci assessed. The mutated seeds were randomly allocated to genotypes carrying a heterozygous allele for the given loci (Aa, Bb, Cc) and randomly subtracted from genotypes carrying the wild type allele (aa, bb, cc). The distribution among the cells was random.

$$MutSeeds_{Locus} = SeedsNew_{Locus,Wt} * MutRate \tag{4.17}$$

Seeds are believed to distribute across cell borders to a limited extent. For this purpose

a von-Neumann distribution into neighboring cells is assumed. All newly formed seeds ($MutSeeds$) are added to the germination layer of the soil seedbank.

Genotype	ABC	aBC	AbC	abC	ABc	aBc	Abc	abc
AABBCC	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
AABBCc	0.50	0.00	0.00	0.00	0.50	0.00	0.00	0.00
AABBcc	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
AABbCC	0.50	0.00	0.50	0.00	0.00	0.00	0.00	0.00
AABbCc	0.25	0.00	0.25	0.00	0.25	0.00	0.25	0.00
AABbcc	0.00	0.00	0.00	0.00	0.50	0.00	0.50	0.00
AAbbCC	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00
AAbbCc	0.00	0.00	0.50	0.00	0.00	0.00	0.50	0.00
AAbbcc	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
AaBBCC	0.50	0.50	0.00	0.00	0.00	0.00	0.00	0.00
AaBBCc	0.25	0.25	0.00	0.00	0.25	0.25	0.00	0.00
AaBBcc	0.00	0.00	0.00	0.00	0.50	0.50	0.00	0.00
AaBbCC	0.25	0.25	0.25	0.25	0.00	0.00	0.00	0.00
AaBbCc	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12
AaBbcc	0.00	0.00	0.00	0.00	0.25	0.25	0.25	0.25
AabbCC	0.00	0.00	0.50	0.50	0.00	0.00	0.00	0.00
AabbCc	0.00	0.00	0.25	0.25	0.00	0.00	0.25	0.25
Aabbcc	0.00	0.00	0.00	0.00	0.00	0.00	0.50	0.50
aaBBCC	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
aaBBCc	0.00	0.50	0.00	0.00	0.00	0.50	0.00	0.00
aaBBcc	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00
aaBbCC	0.00	0.50	0.00	0.50	0.00	0.00	0.00	0.00
aaBbCc	0.00	0.25	0.00	0.25	0.00	0.25	0.00	0.25
aaBbcc	0.00	0.00	0.00	0.00	0.00	0.50	0.00	0.50
aabbCC	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
aabbCc	0.00	0.00	0.00	0.50	0.00	0.00	0.00	0.50
aabbcc	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00

Table 4.7: Value for HP_{Form} showing the contribution of every genotype to every haplotype

Random Factors

Two random factors are introduced into the model. One representing the conditions in fall ($Konst_{Fall}$) and the other the conditions in spring ($Konst_{Spring}$). Both factors are random numbers drawn from the interval [0.8,1.2] with equal probability. The variables influenced by the two factors are shown in table 4.8

$Konst_{Autumn}$	$Konst_{Spring}$
paG (Germination) Survival of herbicide application (C_0-C_3)	Survival of herbicide application (C_4-C_5)

Table 4.8: Variables being influenced by the two random factors $Konst_{Fall}$ and $Konst_{Spring}$.

Testing and adjusting the parameters of the model

As the complexity of the models enables various combinations of parameters, a screening of suitable parameters was carried out. A total of 29 fields with more than ten years of field history was used and various combinations of parameters applied to them. The assessment of the suitability of a parameter was done by visually comparing the predicted results for a particular field to the data obtained in the field, greenhouse and laboratory. Every model run consisted of 25 replicates from which average values for the plants surviving in every year ($MatPfl_t$) were calculated. The most suitable set of parameters was then picked and tested with 128 replicates.

Final Parameter estimates

Parameterization was done using values from table 4.9.

Parameter	Value	Description
FieldHeight	10	Number of cells of CA in y-Dimension
FieldWidth	10	Number of cells of CA in x-Dimension
paG	0.5	Germination rate
pS	0.25	Survival rate of seeds in the soil from t to t+1
Amax	3500	maximum number of seeds produced per *A. myosuroides* plant
ResistanceFreqA	0.00001	Initial Frequency of resistance to LociA
ResistanceFreqBA	0.3	Initial Frequency of resistance to LociB
ResistanceFreqC	0.1	Initial Frequency of resistance to LociC
MutRate	0.00001	Spontaneous mutation rate for LociA, LociB, LociC
SqmPerCell	100	sqm per individual cell of the CA

Table 4.9: Parameters used in the simulation and their corresponding values.

Dimensions for the CA are rather small, representing only 1ha. Dunker et al. (2002) uses a much larger CA though the values were chosen here to test various different combinations and keep computing times at a reasonable level to achieve the goal of developing a practical application.

Values for Germination Rate (paG) were chosen after initial testing of the model with real life examples (data not shown). Values for this parameter appear highly variable. Naylor et al. (1972) reported that 60-70% of the germinated seeds were from the previous

season. Colbach et al. (2002) reported values between 38-70% for the germination rate of *A. myosuroides* . Considerably lower values were reported by Doyle et al. (1986) and Zwerger (1993) assuming levels of <20% with a high annual effect detected by Zwerger (1993).

A_{max} values were set to 3500 which is lower than the A_{max} of 10000 reported by Dunker et al. (2002). Values for this parameter are highly variable as shown in section 1.2.1 and might depend on several factors such as soil and nutrient supply among others. Values for ResistanceFreqA and MutRate are within the range reported by Jasieniuk et al. (1996). Values for ResistanceFreqB and ResistanceFreqC were empirically defined by randomly testing different combinations.

4.3 Results

4.3.1 Supervised Learning

The test data set of 36 fields derived from the 106 fields used in chapter 3 was analyzed with the trained random forest model developed from 70 fields. Figure 4.3 shows the variable importance for both classification accuracy and Gini Index. The figure indicates that the variable (SV_UW) ranks top for both measures and is therefore of highest influence. SCrops follows in second position and ALOMY_GrpB or WCereals are in third position. These variables were ranked of highest importance for an accurate prediction. Among the soil variables Soil2 is of highest importance for accuracy of prediction. In general, variables describing the crop rotation seem of higher importance for an accurate prediction than variables describing the herbicide regime.

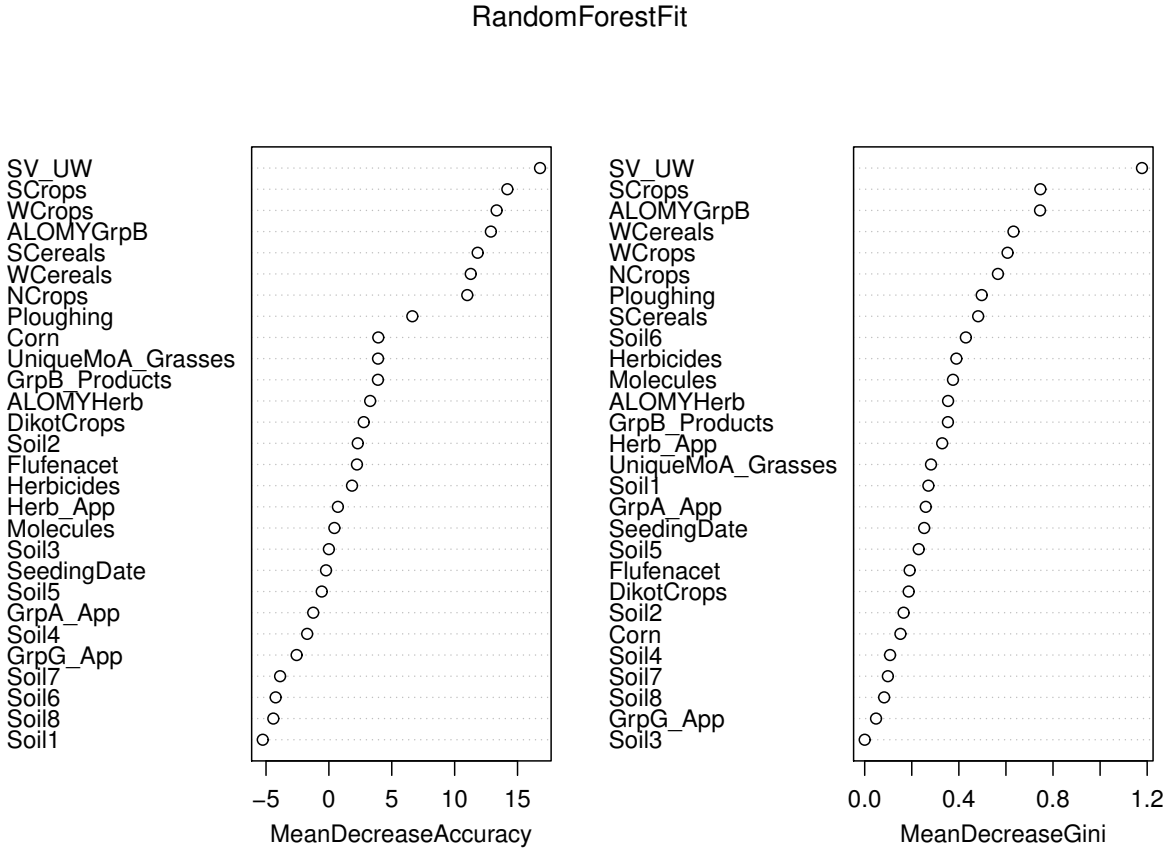


Figure 4.3: Variance importance plot ranking the variables by mean accuracy decrease (left) and mean decrease gini (Gini Index, right)

Data to test the algorithm consists of 16, 7 and 13 fields that were classified as R, I and S respectively. Table 4.10 shows the real output compared to the predicted output.

Among the 36 fields classified 13% were clearly classified wrongly (S being predicted as R).

However, there are no cases in which an R case was predicted as something else. The prediction of the intermediate cases into R and S shows that this group is very difficult to predict accurately despite being the most important if one focuses on early prediction of resistance.

Atlantis Status	Prediction Standard		Prediction Modified				
	R	S	S	S/I	I	I/R	R
S	4	9	8	2	1	1	1
I	4	3	1	2	1	2	1
R	16				3	3	10

Table 4.10: Comparison of Atlantis Status to the predicted status of 36 fields tested. Prediction Standard represents the outcome of the algorithm without adjustments. Prediction Modified presents the estimation with adjustments from table 4.12.

The sampling frequency for the R group was taken and plotted for the different Atlantis statuses of the test data set (figure 4.4). This frequency displays the portion of the 1500 trees used to train the random forest model that classified a given sample as R. Figure 4.4 shows that even the sensitive group is predicted as R in between 24% and 51% of the cases for the majority of data points. Considering the quantiles at 25% and 75% an almost perfect separation between the groups was achieved (table 4.11). Besides the already described outliers, the separation between R and S was very good.

Atlantis Status	Q10	Q25	Mean	Median	Q75	Q90
S	0.24	0.28	0.42	0.36	0.51	0.67
I	0.42	0.45	0.55	0.54	0.67	0.69
R	0.63	0.67	0.74	0.73	0.81	0.86

Table 4.11: 10% quantile, 25% quantile, 75% quantile, 90% quantile, mean and median calculated for the frequency of being predicted as R for the three Atlantis Groups

Based on values from table 4.11 and figure 4.4 borders for the prediction groups were defined (table 4.12). The definition of these groups is necessary to have guidelines on when to consider a sample as resistant. Based on these new groups the classification by majority that was used in table 4.10 (left) was discarded and the frequency of votes with the new groups considered (table 4.10, right).

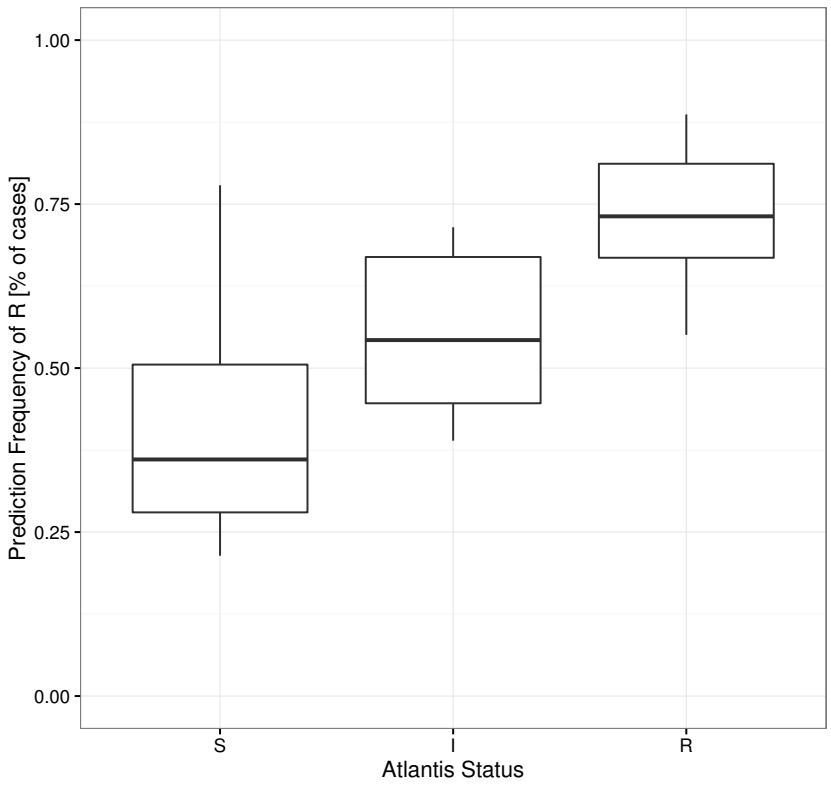


Figure 4.4: Frequency of prediction of resistance for 36 fields using a trained random forest model. The test dataset consists out of of 16, 7 and 13 fields that were classified as R, I and S respectively.

Category	Min	Max
S	0	0.414
S/I	0.415	51
I	0.52	0.66
I/R	0.66	0.69
R	0.7	1

Table 4.12: Derived categories with their limits of values for predicting the resistance status to Atlantis WG .

4.3.2 Simulation Model

A simulation model was used as a second approach to predict the resistance status in the field. The parameters of the model were adopted based on literature findings and own adjustments of parameters. Tests of the model with actual data from fields were carried out to adopt the model for the best possible fit. The field Z038, which was the starting field for location Z is described below. Table 4.13 shows the field history for this field. The farmer applied an ALS-inhibitor five times in four different years before he noticed resistance. 2009 was therefore the last time this field was treated with an ALS-inhibitor following which the farmer complained about the low efficacy of the product. Ploughing was not carried out until 2013. The field contains soils that were grouped into cluster 4 and cluster 2 with 79% and 21% coverage respectively, as represented by the CA (figure 4.2). Table 4.13 shows furthermore that the farmer was following an oilseed rape-2x winter wheat-winter barley crop rotation before introducing corn in 2013. Summer wheat was grown in 2012 but as a deviation from the routine due to the strong winter as indicated earlier.

Year	Crop	Seeding Date	Tillage	Flufenacet	PRE (Other)	FOP (F)	FOP (S)	DIM (F)	DIM (S)	ALS (F)	ALS (S)	Alt (F)	Alt (S)	Glyphosate
2004	TRZAW	normal	Cultivator							X				
2005	TRZAW	normal	Cultivator		X					X	X			
2006	HORVW	normal	Cultivator	X		X	X							
2007	BRSNW	normal	Cultivator		X	X								
2008	TRZAW	normal	Cultivator								X			
2009	TRZAW	normal	Cultivator	X			X				X			
2010	HORVW	normal	Cultivator	X			X							X
2011	BRSNW	normal	Cultivator		X			X						
2012	TRZAS	normal	Cultivator											X
2013	ZEAMX	normal	Plough	X										X
2014	ZEAMX	normal	Plough	X										X
2015	TRZAW	normal	Plough	X									X	

Table 4.13: Field History of Z038 for the years 2004-2015. Data was translated into the corresponding applications with F and S denoting fall and spring application respectively. Variables are explained in table 4.16

.

The observed values for this field are shown in table 4.14 and imply that between 2010 and 2012 the highest infestation values occurred. These were at levels effecting yield but dropped after the introduction of corn to the rotation. However, in all years the analysis of field survivors showed low levels of control with Atlantis WG in greenhouse tests and a high frequency of P197X.

Year	Infestation	AtlantisCont	MaisterCont	P197X	W574X
2010	4	11	73		
2011	4	8	85	77	0
2012	4	12	85	86	0
2013	2	36	88	40	0
2014	1	14	50	44	35

Table 4.14: Observed results for Z038 between 2010 and 2015 for Infestation, Atlantis WG control (AtlantisCont, (%)), MaisTer fluessig control (MaisterCont, (%)) and the two target site mutations assessed (P197X, W574X)

The simulation shows for Z038 that the level of control was very high in the first two simulated years when ALS-inhibitors were applied to winter wheat and infestation levels at the end of the season were simulated to be low. The simulated infestation levels increased in the following two years when winter barley and oilseed rape where grown. Interesting to note is that when barley was grown, two applications with a FOP herbicide were carried out in fall and spring by the farmer, suggesting high infestation levels at the time of spraying. When an ALS-inhibitor was applied the fourth time (third year) in 2008, more than 50% of the plants were simulated to be resistant. This level was further increased with the fifth (and last) application of an ALS-inhibitor in 2009. After that, the portion of homozygous (AA), heterozygous (Aa) and wildtype (aa) plants remains almost the same with different numbers of plants resulting from the application. Note that these values represent the sum of all genotypes carrying either an AA, Aa or aa allele. The two highest numbers of *A. myosuroides* were simulated for the years 2011 and 2012 while levels from 2013 on were lower. This fits well the observation taken in the field table 4.13. The large number of plants observed in 2011 however was not very pronounced in the simulation. The total number of plants are however difficult to compare as the simulation predicts actual numbers while groups of infestation ranging from 0–5 were assessed in the field. Furthermore the observed frequency for P197X ranging from 40% to 86% are well approximated by the simulation.

The simulation shows furthermore an interesting aspect in the development of resistance. While the control of *A. myosuroides* was very high in the first two years of application (2004, 2005) it was not satisfying in the last two (2008, 2009). In the two years in between, a considerable enrichment of ALS resistant seeds occurred in the absence of an ALS application. The seeds were propagated because they contained additional resis-

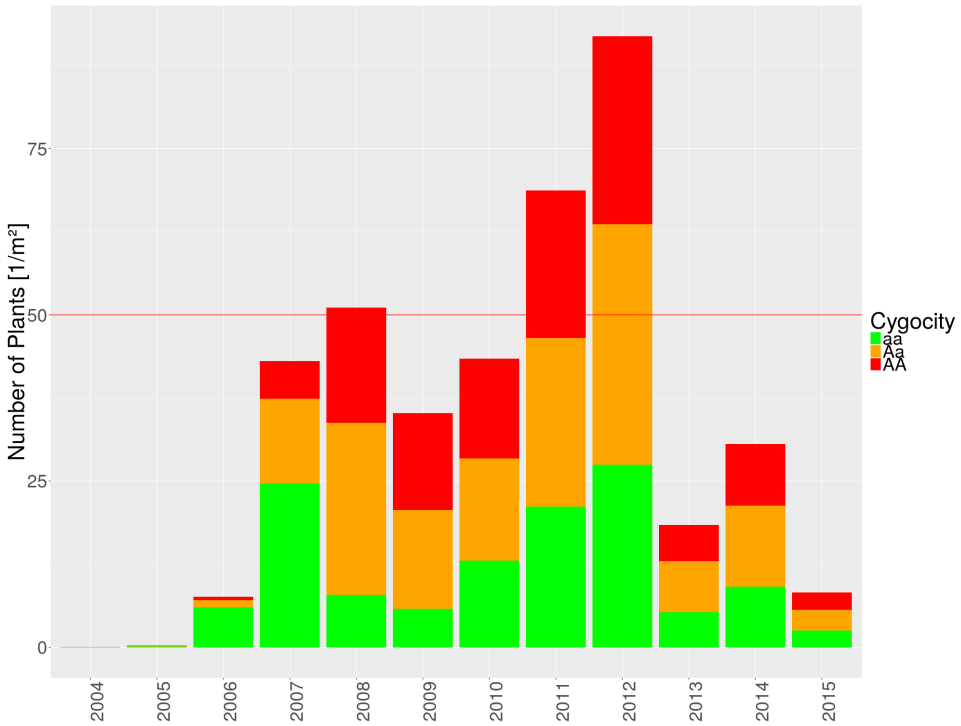


Figure 4.5: Simulation Output for field "Z038" with total number of plants and the respective portion of homozygous(AA), heterozygous (Aa) and wildtype (aa) plants

tance alleles to either FOP and/or DIM (Locus B and/or C) herbicides that were applied by the farmer (table 4.15). Random mating across all survivors leads therefore to a faster increase in the number (not the frequency) of one resistant allele if *A. myosuroides* can no longer be sufficiently controlled in one crop.

The influence of the soil is observable in the simulation as resistant genotypes are mostly found in those areas of the CA that are reported as having heavier soils (e.g. 2008-2012, compared to figure 4.2). This implies that soils with a higher carrying capacity serve as a starting point in the field for resistance development. The opposite is also true: the simulation indicates that the number of resistant genotypes is still higher in the areas with a higher carrying capacity.

Genotype	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
aabbcc	0.02	0.04	0.00	0.70	0.84	0.00	0.00	0.33	1.85	0.30	0.66	0.21
aabbCc	0.01	0.01	0.90	3.04	0.90	0.73	1.53	2.95	3.02	0.61	1.07	0.30
aabbCC	0.00	0.00	0.07	1.99	0.79	0.63	1.52	3.04	3.13	0.53	0.92	0.26
aaBbcc	0.02	0.04	3.07	5.20	0.96	0.77	1.58	0.71	2.52	0.52	0.97	0.28
aaBbCc	0.01	0.00	0.77	3.58	0.98	0.80	1.79	3.61	4.11	0.91	1.44	0.36
aaBbCC	0.00	0.00	0.06	1.92	0.85	0.74	1.74	3.46	4.10	0.80	1.24	0.33
aaBBcc	0.01	0.00	0.90	4.41	0.94	0.75	1.61	0.69	2.21	0.37	0.74	0.23
aaBBCc	0.00	0.00	0.23	2.70	0.92	0.79	1.77	3.32	3.32	0.68	1.12	0.31
aaBBCC	0.00	0.00	0.01	1.09	0.69	0.60	1.51	3.07	3.20	0.55	0.94	0.27
Aabbcc	0.00	0.11	0.00	0.29	2.67	0.00	0.00	0.55	2.35	0.49	0.94	0.27
AabbCc	0.00	0.02	0.14	1.72	2.90	1.82	1.81	3.42	3.96	0.88	1.42	0.36
AabbCC	0.00	0.00	0.02	0.78	2.29	1.74	1.86	3.51	4.10	0.80	1.24	0.32
AaBbcc	0.00	0.08	0.61	2.91	3.51	1.89	1.84	0.97	3.25	0.78	1.29	0.34
AaBbCc	0.00	0.01	0.09	2.00	3.60	1.98	2.03	4.41	5.56	1.23	1.90	0.44
AaBbCC	0.00	0.00	0.01	0.67	2.52	1.88	2.02	4.08	5.52	1.09	1.64	0.39
AaBBcc	0.00	0.02	0.22	2.70	3.38	1.90	1.92	0.95	2.83	0.59	1.02	0.29
AaBBCc	0.00	0.00	0.03	1.46	3.08	1.96	2.05	3.94	4.40	0.96	1.49	0.37
AaBBCC	0.00	0.00	0.00	0.27	1.97	1.71	1.86	3.55	4.21	0.82	1.26	0.33
AAbbcc	0.00	0.02	0.00	0.07	1.86	0.00	0.00	0.38	1.91	0.31	0.68	0.21
AAbbCc	0.00	0.00	0.06	0.65	1.93	1.79	1.76	3.06	3.11	0.63	1.09	0.30
AAbbCC	0.00	0.00	0.01	0.31	1.35	1.66	1.80	3.16	3.21	0.55	0.93	0.27
AABbcc	0.00	0.01	0.27	1.58	2.59	1.88	1.79	0.78	2.61	0.54	0.99	0.28
AABbCc	0.00	0.00	0.04	0.75	2.46	1.95	1.98	3.78	4.26	0.94	1.47	0.37
AABbCC	0.00	0.00	0.00	0.21	1.49	1.83	1.97	3.60	4.21	0.82	1.26	0.33
AABBcc	0.00	0.00	0.11	1.51	2.55	1.88	1.86	0.75	2.29	0.39	0.76	0.23
AABBCc	0.00	0.00	0.01	0.43	2.07	1.93	2.00	3.46	3.43	0.71	1.14	0.31
AABBCC	0.00	0.00	0.00	0.09	1.00	1.62	1.80	3.19	3.28	0.57	0.96	0.27
Total	0.07	0.36	7.63	43.03	51.09	35.23	43.40	68.72	91.95	18.37	30.58	8.23

Table 4.15: Observed average numbers for every genotype surviving for each simulated year for field Z038.

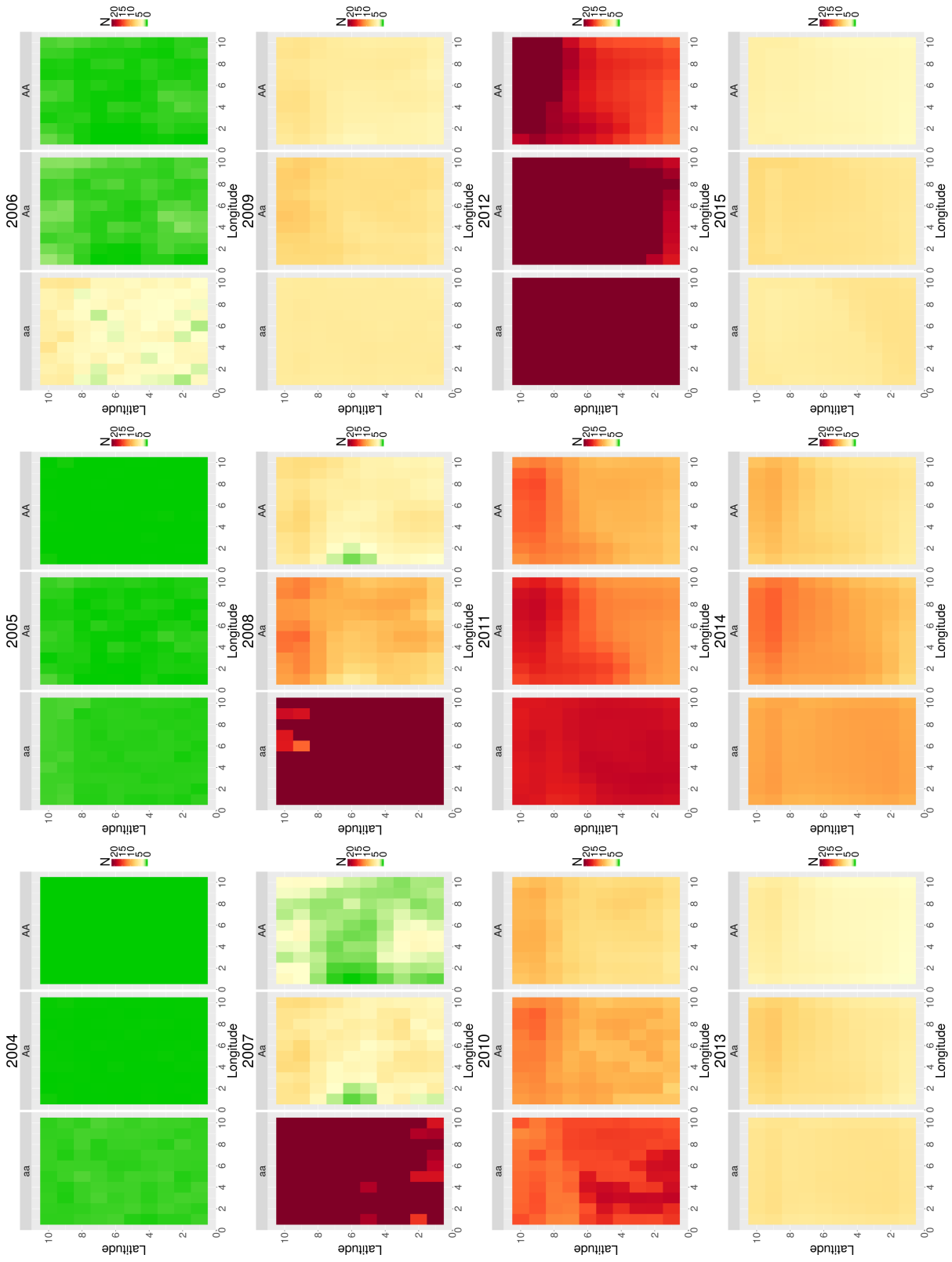


Figure 4.6: Annual Number and Distribution of homozygous(AA), heterozygous (Aa) and wildtype (aa) plants within the field simulated by CA

4.3.3 Prediction Outcomes

Both predictions by random forest and the simulation model together with actual observational date are presented on the next pages. Each page contains one example. 16 out of the 36 fields from the test data set of the random forest algorithm had 10 years or more of field history. They are presented below to compare both approaches side by side.

The layout of the following pages is as following:

Title: The chosen field and the corresponding status for Atlantis WG as defined.

Observational Data: Data obtained from field sampling, greenhouse analysis (Control by Atlantis WG and MaisTer fluessig [%]) and laboratory analysis (P197X, W574X [%]).

Random Forest: Prediction by the random forest algorithm. Predicted Status shows the prediction of the five groups derived from table 4.12. Predicted Frequency shows the frequency among the 1500 randomly created trees that group this sample into R.

Simulation Model: Outcome of the simulation model by year. The outcome is separated into sensitive for ALS (aa), heterozygous resistant (Aa) and homozygous resistant (AA). Values present average values after 128 runs of the simulation. Those of the 27 genotypes containing either AA, Aa or aa were summed up to derive the values presented.

Field History: Measures applied by the farmer on the corresponding field. Data for herbicide treatments is divided into a fall application (F) and a spring application (S). Herbicide applications were coded as in table 4.16

Variable	Explanation
FOP	FOP/DEN ACCase-inhibitor
DIM	DIM ACCase-inhibitor
ALS	ALS-inhibitor
Flufenacet	Flufenacet application
PRE_Other	Other soil active herbicide active on *A. myosuroides*
Glphosate	Glyposate application

Table 4.16: Variable Explanation for Field History tables provided

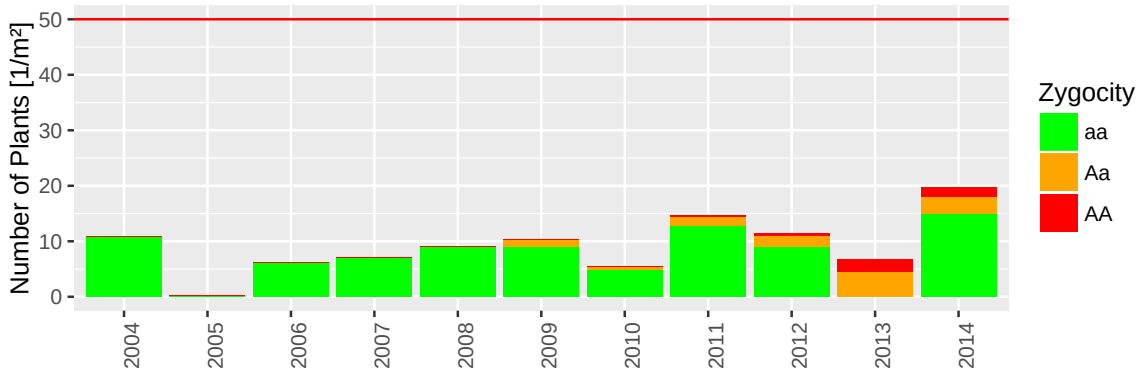
Example H012: (Atlantis Status S)

Observational Data

FieldID	Year	Infestation	AtlantisCont	MaisterCont	P197X	W574X
H012	2010	0				
H012	2011	1	100	100	0	0
H012	2012	1			0	0

Random Forest
Predicted Status: S
Predicted Frequency (R): 0.27

Simulation Model



Field History

Year	Crop	Seeding Date	Tillage	Flufenacet	PRE (Other)	FOP (F)	FOP (S)	DIM (F)	DIM (S)	ALS (F)	ALS (S)	Alt (F)	Alt (S)	Glyphosate
2004	BRSNW	normal	Plough			X								
2005	TRZAW	normal	Cultivator								X			
2006	AVESA	normal	Plough								X			
2007	HORVW	normal	Plough		X	X								
2008	BRSNW	normal	Plough					X						
2009	TRZAW	normal	Cultivator		X		X			X				
2010	AVESA	normal	Plough								X			
2011	HORVW	normal	Plough	X		X								
2012	BRSNW	late	Plough		X			X						
2013	TRZAW	normal	Cultivator								X			
2014	HORVW	normal	Plough	X										

Figure 4.7: Comparison of the predicted resistance status by random forest and simulation model to observational data for field H012. Field Management by the farmer indicates information about the crop, tillage and herbicide regime.

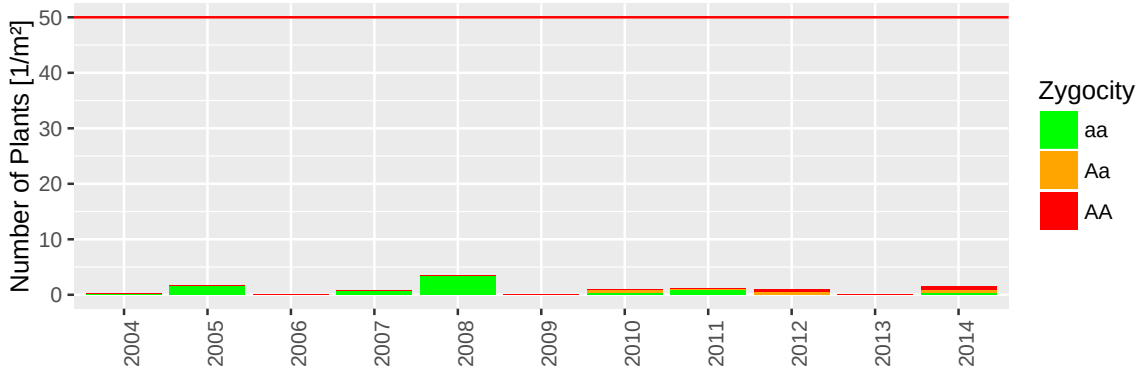
Example H289: (Atlantis Status I)

Observational Data

FieldID	Year	Infestation	AtlantisCont	MaisterCont	P197X	W574X
H289	2013	2	68	38	50	69
H289	2014	2	20	43		

Random Forest
Predicted Status: S
Predicted Frequency (R): 0.39

Simulation Model



Field History

Year	Crop	Seeding Date	Tillage	Flufenacet	PRE (Other)	FOP (F)	FOP (S)	DIM (F)	DIM (S)	ALS (F)	ALS (S)	Alt (F)	Alt (S)	Glyphosate
2004	TRZAW	normal	Disc								X			
2005	TTLWI	normal	Disc	X			X							
2006	HORVW	normal	Plough				X						X	
2007	BRSNW	normal	Plough		X			X						
2008	TRZAW	normal	Disc							X				
2009	HORVW	normal	Plough	X			X						X	
2010	TTLWI	normal	Disc		X					X				
2011	BRSNS	normal	Plough		X			X	X					
2012	TRZAW	normal	Disc	X	X						X			
2013	TTLWI	normal	Plough		X					X	X		X	
2014	TRZAW	normal	Plough	X										

Figure 4.8: Comparison of the predicted resistance status by random forest and simulation model to observational data for field H289. Field Management by the farmer indicates information about the crop, tillage and herbicide regime.

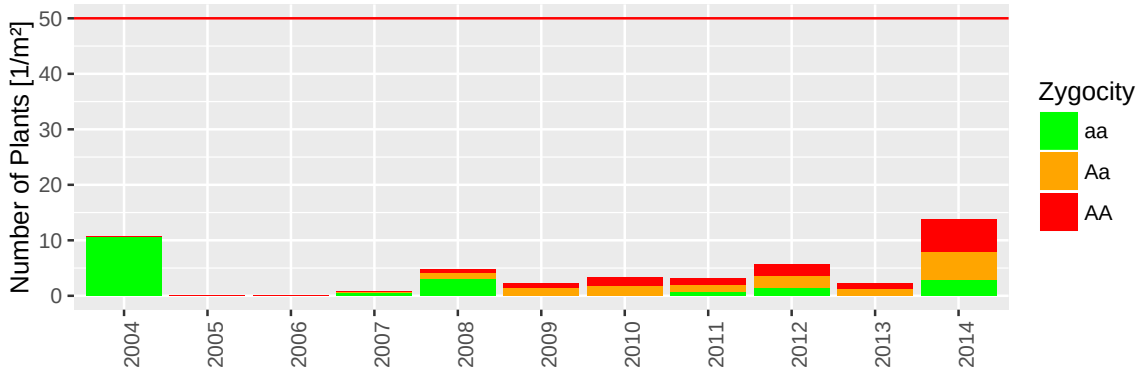
Example H362: (Atlantis Status R)

Observational Data

FieldID	Year	Infestation	AtlantisCont	MaisterCont	P197X	W574X
H362	2014	2	19	56		

Random Forest
Predicted Status: R
Predicted Frequency (R): 0.75

Simulation Model



Field History

Year	Crop	Seeding Date	Tillage	Flufenacet	PRE (Other)	FOP (F)	FOP (S)	DIM (F)	DIM (S)	ALS (F)	ALS (S)	Alt (F)	Alt (S)	Glyphosate
2004	BRSNW	late	Plough		X	X								
2005	TRZAW	normal	Cultivator								X			
2006	TTLWI	normal	Cultivator								X			
2007	HORVW	normal	Cultivator	X			X							
2008	BRSNW	normal	Cultivator		X	X								
2009	TRZAW	normal	Cultivator								X			
2010	TTLWI	normal	Cultivator								X			
2011	HORVW	normal	Cultivator	X			X							
2012	ZEAMX	normal	Cultivator								X			
2013	TRZAW	late	Plough								X			
2014	TTLWI	normal	Cultivator								X			

Figure 4.9: Comparison of the predicted resistance status by random forest and simulation model to observational data for field H362. Field Management by the farmer indicates information about the crop, tillage and herbicide regime.

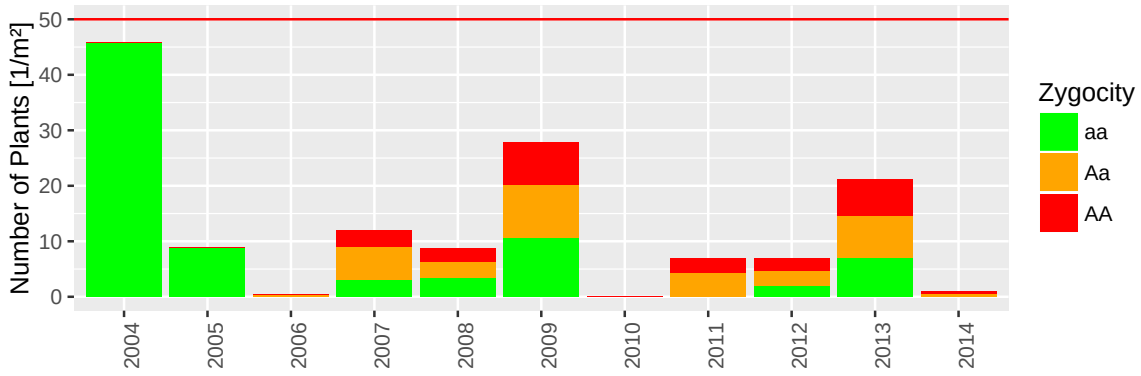
Example M001: (Atlantis Status R)

Observational Data

FieldID	Year	Infestation	AtlantisCont	MaisterCont	P197X	W574X
M001	2011	4	15	32	0	17
M001	2012	3	9	11	0	0
M001	2014	3	12	7		

Random Forest
Predicted Status: R
Predicted Frequency (R): 0.77

Simulation Model



Field History

Year	Crop	Seeding Date	Tillage	Flufenacet	PRE (Other)	FOP (F)	FOP (S)	DIM (F)	DIM (S)	ALS (F)	ALS (S)	Alt (F)	Alt (S)	Glyphosate
2004	HORVW	normal	Plough	X										
2005	BRSNW	normal	Cultivator		X	X	X							
2006	TRZAW	normal	Cultivator							X	X			
2007	TRZAW	normal	Disc		X					X				
2008	HORVW	normal	Disc	X			X							
2009	BRSNW	normal	Disc		X			X						
2010	TRZAW	normal	Cultivator		X		X			X	X			
2011	TRZAW	normal	Cultivator	X							X			
2012	HORVW	normal	Plough	X										
2013	BRSNW	normal	Cultivator		X	X		X						
2014	TRZAW	normal	Cultivator	X							X		X	

Figure 4.10: Comparison of the predicted resistance status by random forest and simulation model to observational data for field M001. Field Management by the farmer indicates information about the crop, tillage and herbicide regime.

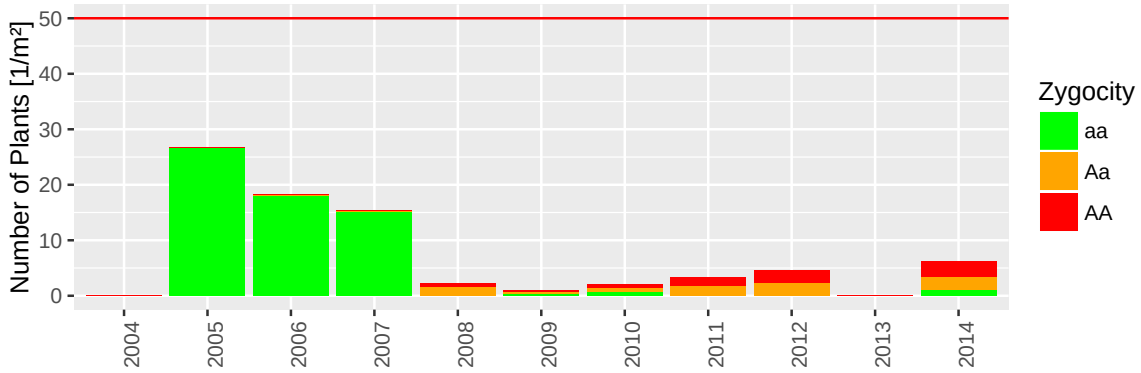
Example M007: (Atlantis Status R)

Observational Data

FieldID	Year	Infestation	AtlantisCont	MaisterCont	P197X	W574X
M007	2011	1	57	55	0	33
M007	2012	5	9	19	0	100
M007	2013	1	18	14	0	76
M007	2014	1	0	25	27	70

Random Forest
Predicted Status: I
Predicted Frequency (R): 0.66

Simulation Model



Field History

Year	Crop	Seeding Date	Tillage	Flufenacet	PRE (Other)	FOP (F)	FOP (S)	DIM (F)	DIM (S)	ALS (F)	ALS (S)	Alt (F)	Alt (S)	Glyphosate
2004	TRZAW	normal	Cultivator								X			
2005	HORVW	normal	Plough	X										
2006	BRSNW	normal	Cultivator		X	X								
2007	TRZAW	normal	Disc		X					X				
2008	TRZAW	normal	Disc								X			
2009	HORVW	normal	Plough	X			X							
2010	BRSNW	normal	Cultivator		X	X			X					
2011	TRZAW	normal	Disc								X			
2012	TTLWI	normal	Disc								X			
2013	HORVW	normal	Plough	X			X						X	
2014	BRSNW	normal	Disc		X			X						

Figure 4.11: Comparison of the predicted resistance status by random forest and simulation model to observational data for field M007. Field Management by the farmer indicates information about the crop, tillage and herbicide regime.

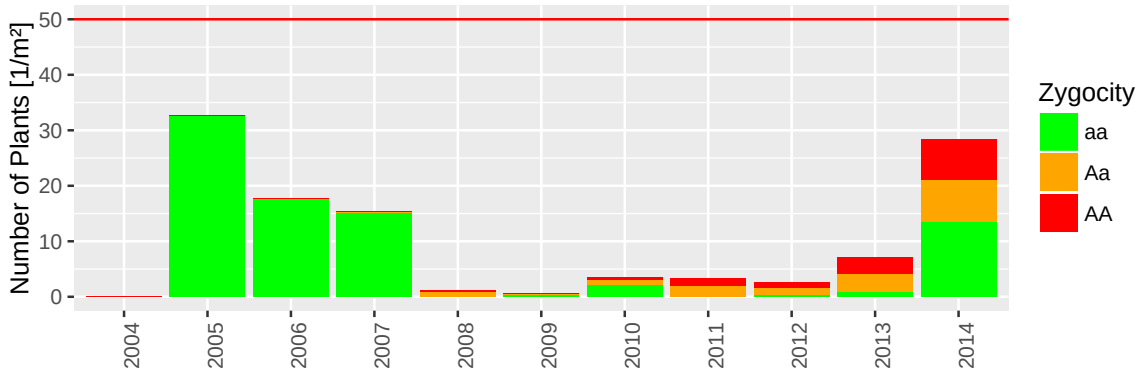
Example M017: (Atlantis Status R)

Observational Data

FieldID	Year	Infestation	AtlantisCont	MaisterCont	P197X	W574X
M017	2011	1	48		0	52
M017	2013	2	17	60	50	94
M017	2014	1	11	23	82	82

Random Forest
Predicted Status: I/R
Predicted Frequency (R): 0.66

Simulation Model



Field History

Year	Crop	Seeding Date	Tillage	Flufenacet	PRE (Other)	FOP (F)	FOP (S)	DIM (F)	DIM (S)	ALS (F)	ALS (S)	Alt (F)	Alt (S)	Glyphosate
2004	TRZAW	normal	Cultivator								X			
2005	HORVW	normal	Plough	X										
2006	BRSNW	normal	Cultivator		X	X								
2007	TRZAW	normal	Disc		X					X				
2008	TRZAW	normal	Disc								X			
2009	HORVW	normal	Plough	X			X							
2010	BRSNW	normal	Cultivator		X	X			X					
2011	TRZAW	normal	Disc								X			
2012	TRZAW	late	Cultivator		X		X			X				
2013	TRZAW	normal	Cultivator	X						X				
2014	BRSNW	normal	Disc		X			X						

Figure 4.12: Comparison of the predicted resistance status by random forest and simulation model to observational data for field M017. Field Management by the farmer indicates information about the crop, tillage and herbicide regime.

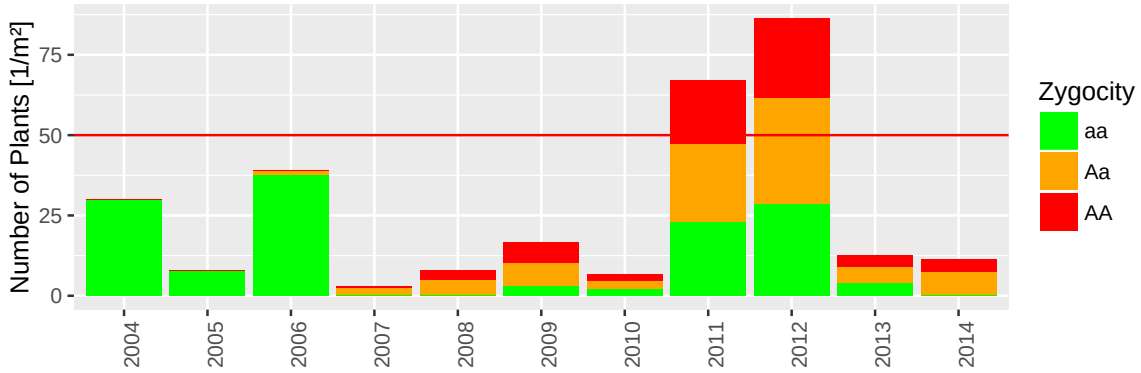
Example M018: (Atlantis Status R)

Observational Data

FieldID	Year	Infestation	AtlantisCont	MaisterCont	P197X	W574X
M018	2011	3	50	68	0	28
M018	2012	1	33	54	0	47
M018	2013	2	8	30	81	81
M018	2014	1	27	47	0	73

Random Forest
Predicted Status: R
Predicted Frequency (R): 0.77

Simulation Model



Field History

Year	Crop	Seeding Date	Tillage	Flufenacet	PRE (Other)	FOP (F)	FOP (S)	DIM (F)	DIM (S)	ALS (F)	ALS (S)	Alt (F)	Alt (S)	Glyphosate
2004	HORVW	normal	Cultivator	X										
2005	HORVW	normal	Cultivator				X							
2006	BRSNW	normal	Disc		X	X								
2007	TRZAW	normal	Disc							X	X			
2008	TRZAW	normal	Disc								X			
2009	TTLWI	normal	Disc		X					X				
2010	TTLWI	normal	Disc				X						X	
2011	BRSNW	normal	Disc		X									
2012	HORVS	normal	Disc											X
2013	ZEAMX	normal	Plough								X			
2014	TRZAW	late	Plough	X							X		X	

Figure 4.13: Comparison of the predicted resistance status by random forest and simulation model to observational data for field M018. Field Management by the farmer indicates information about the crop, tillage and herbicide regime.

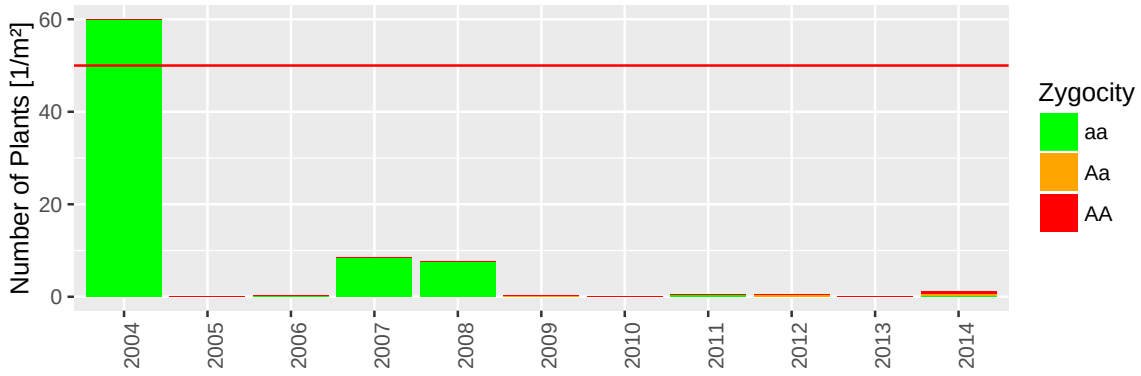
Example M027: (Atlantis Status R)

Observational Data

FieldID	Year	Infestation	AtlantisCont	MaisterCont	P197X	W574X
M027	2012	3			0	8
M027	2013	3	13	17	0	82
M027	2014	2	40	29		

Random Forest
Predicted Status: I
Predicted Frequency (R): 0.61

Simulation Model



Field History

Year	Crop	Seeding Date	Tillage	Flufenacet	PRE (Other)	FOP (F)	FOP (S)	DIM (F)	DIM (S)	ALS (F)	ALS (S)	Alt (F)	Alt (S)	Glyphosate
2004	AVESA	normal	Plough								X			
2005	HORVW	normal	Cultivator	X	X									
2006	TTLWI	normal	Cultivator								X			
2007	HORVW	normal	Plough				X						X	
2008	TRZAS	normal	Cultivator											
2009	TRZAW	normal	Cultivator							X				
2010	TRZAW	normal	Cultivator		X					X	X			
2011	HORVW	late	Plough				X						X	
2012	HORVW	normal	Disc	X			X							
2013	TRZAW	normal	Disc								X			
2014	TTLWI	normal	Plough	X							X			

Figure 4.14: Comparison of the predicted resistance status by random forest and simulation model to observational data for field M027. Field Management by the farmer indicates information about the crop, tillage and herbicide regime.

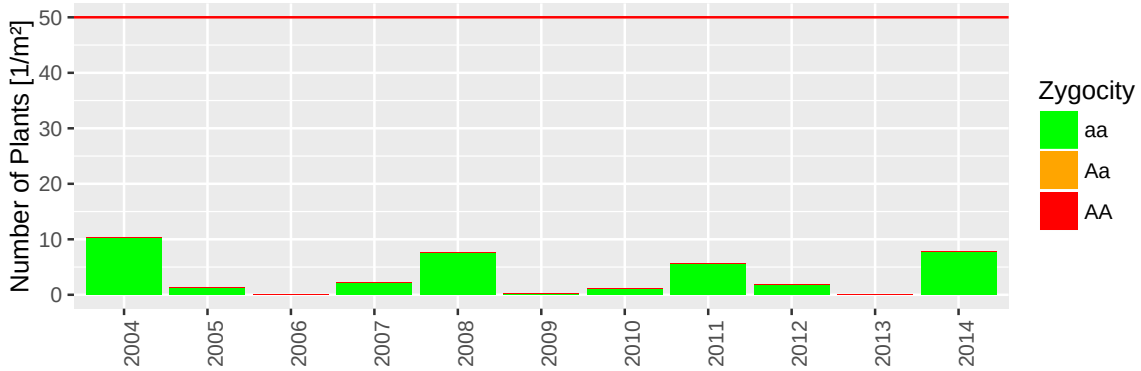
Example Z002: (Atlantis Status S)

Observational Data

FieldID	Year	Infestation	AtlantisCont	MaisterCont	P197X	W574X
Z002	2010	0				
Z002	2011	1	93	100	0	0
Z002	2012	2	100	100	0	0
Z002	2013	2	93	100	0	0
Z002	2014	2	81	100	3	0

Random Forest
Predicted Status: S
Predicted Frequency (R): 0.31

Simulation Model



Field History

Year	Crop	Seeding Date	Tillage	Flufenacet	PRE (Other)	FOP (F)	FOP (S)	DIM (F)	DIM (S)	ALS (F)	ALS (S)	Alt (F)	Alt (S)	Glyphosate
2004	HORVW	normal	Disc	X			X							
2005	BRSNW	late	Plough		X				X					
2006	TRZAW	normal	Cultivator				X				X			
2007	HORVW	normal	Plough	X			X							
2008	BRSNW	normal	Plough		X									
2009	TRZAW	normal	Disc							X	X			
2010	HORVS	normal	Plough										X	
2011	HORVW	normal	Disc	X		X								
2012	BRSNW	normal	Plough		X			X						
2013	TRZAW	normal	Disc				X				X			
2014	HORVW	normal	Plough	X										

Figure 4.15: Comparison of the predicted resistance status by random forest and simulation model to observational data for field Z002. Field Management by the farmer indicates information about the crop, tillage and herbicide regime.

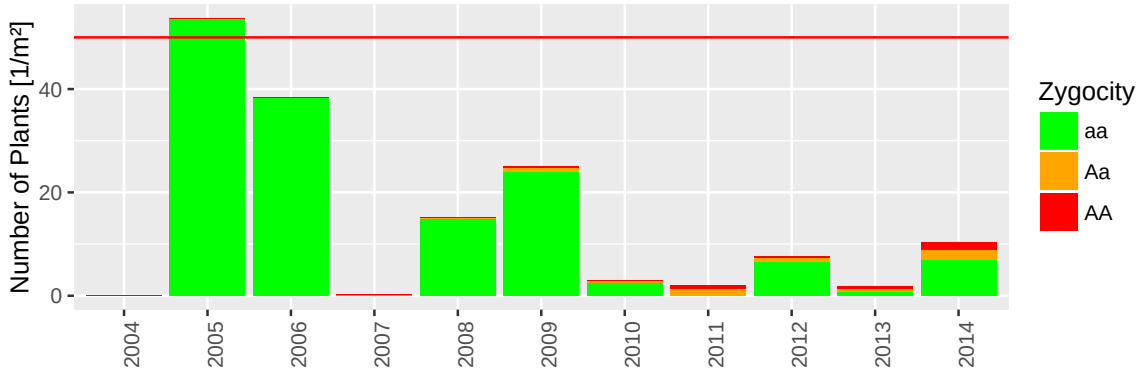
Example Z009: (Atlantis Status S)

Observational Data

FieldID	Year	Infestation	AtlantisCont	MaisterCont	P197X	W574X
Z009	2011	1	94		0	0
Z009	2012	1	100	100	0	0
Z009	2013	1	100	92	0	0
Z009	2014	1	100	100	0	0

Random Forest
Predicted Status: S
Predicted Frequency (R): 0.21

Simulation Model



Field History

Year	Crop	Seeding Date	Tillage	Flufenacet	PRE (Other)	FOP (F)	FOP (S)	DIM (F)	DIM (S)	ALS (F)	ALS (S)	Alt (F)	Alt (S)	Glyphosate
2004	TRZAW	normal	Disc								X			
2005	HORVW	normal	Plough	X										
2006	BRSNW	normal	Plough		X									
2007	TRZAW	late	Cultivator								X			
2008	HORVS	normal	Disc										X	
2009	HORVW	normal	Disc	X		X								
2010	BRSNW	normal	Plough		X			X						
2011	TRZAW	late	Disc								X			
2012	HORVW	normal	Plough	X		X								
2013	BRSNW	late	Plough		X				X					
2014	TRZAW	normal	Disc	X										

Figure 4.16: Comparison of the predicted resistance status by random forest and simulation model to observational data for field Z009. Field Management by the farmer indicates information about the crop, tillage and herbicide regime.

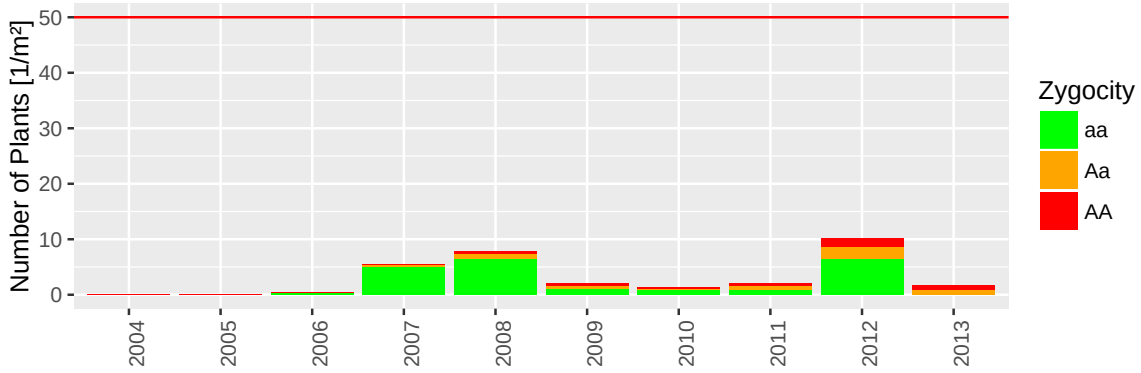
Example Z023: (Atlantis Status I)

Observational Data

FieldID	Year	Infestation	AtlantisCont	MaisterCont	P197X	W574X
Z023	2010	0				
Z023	2011	2	98	100	0	0
Z023	2012	1	94	100	5	0
Z023	2013	1	25	100	56	0
Z023	2014	1	80	100	0	0

Random Forest
Predicted Status: R
Predicted Frequency (R): 0.71

Simulation Model



Field History

Year	Crop	Seeding Date	Tillage	Flufenacet	PRE (Other)	FOP (F)	FOP (S)	DIM (F)	DIM (S)	ALS (F)	ALS (S)	Alt (F)	Alt (S)	Glyphosate
2004	TRZAW	normal	Cultivator								X			
2005	TTLWI	normal	Cultivator		X					X	X			
2006	HORVW	normal	Cultivator	X		X	X							
2007	BRSNW	normal	Cultivator		X	X								
2008	TRZAW	normal	Cultivator							X				
2009	TTLWI	normal	Cultivator				X			X				
2010	HORVW	normal	Plough	X			X							
2011	BRSNW	normal	Plough		X			X						
2012	TRZAS	normal	Disc											
2013	TTLWI	late	Cultivator								X			

Figure 4.17: Comparison of the predicted resistance status by random forest and simulation model to observational data for field Z023. Field Management by the farmer indicates information about the crop, tillage and herbicide regime.

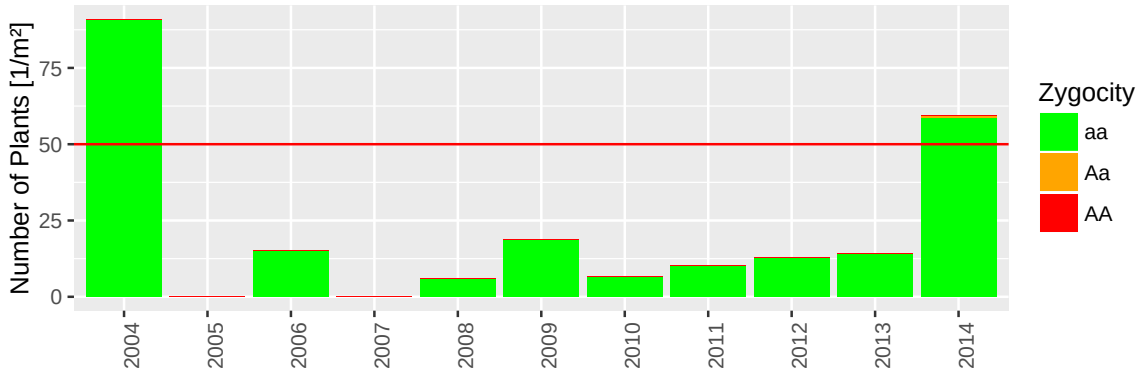
Example Z029: (Atlantis Status R)

Observational Data

FieldID	Year	Infestation	AtlantisCont	MaisterCont	P197X	W574X
Z029	2010	3	65	100		
Z029	2011	1	29	84	36	0
Z029	2012	2	24	82	84	0
Z029	2013	2	59	100	53	0
Z029	2014	1	43	100	9	0

Random Forest
Predicted Status: R
Predicted Frequency (R): 0.7

Simulation Model



Field History

Year	Crop	Seeding Date	Tillage	Flufenacet	PRE (Other)	FOP (F)	FOP (S)	DIM (F)	DIM (S)	ALS (F)	ALS (S)	Alt (F)	Alt (S)	Glyphosate
2004	HORVW	normal	Plough	X										
2005	TRZAW	normal	Plough								X			
2006	HORVW	normal	Plough	X			X							
2007	TRZAW	normal	Plough		X					X	X			
2008	BRSNW	normal	Plough					X						
2009	TRZAW	normal	Cultivator		X					X				
2010	HORVW	normal	Plough	X		X								
2011	BRSNW	normal	Plough		X			X						
2012	HORVS	normal	Cultivator										X	
2013	HORVW	normal	Plough	X										
2014	BRSNW	normal	Plough		X									

Figure 4.18: Comparison of the predicted resistance status by random forest and simulation model to observational data for field Z029. Field Management by the farmer indicates information about the crop, tillage and herbicide regime.

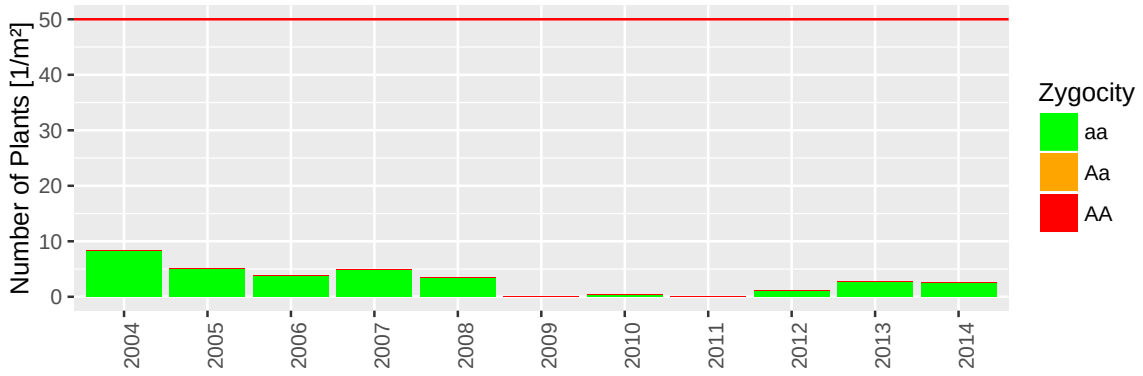
Example Z116: (Atlantis Status S)

Observational Data

FieldID	Year	Infestation	AtlantisCont	MaisterCont	P197X	W574X
Z116	2012	1			0	0
Z116	2013	1	100	100	0	0
Z116	2014	3	97	100		

Random Forest
Predicted Status: I/R
Predicted Frequency (R): 0.69

Simulation Model



Field History

Year	Crop	Seeding Date	Tillage	Flufenacet	PRE (Other)	FOP (F)	FOP (S)	DIM (F)	DIM (S)	ALS (F)	ALS (S)	Alt (F)	Alt (S)	Glyphosate
2004	TRZAW	normal	Cultivator										X	
2005	HORVW	normal	Cultivator										X	
2006	HORVW	normal	Cultivator										X	
2007	TRZAW	normal	Cultivator										X	
2008	HORVW	normal	Cultivator										X	
2009	BRSNW	normal	Cultivator		X			X						
2010	TRZAW	normal	Cultivator								X			
2011	HORVW	normal	Cultivator				X						X	
2012	ZEAMX	normal	Plough										X	
2013	TRZAW	late	Plough								X			
2014	HORVW	normal	Plough	X										

Figure 4.19: Comparison of the predicted resistance status by random forest and simulation model to observational data for field Z116. Field Management by the farmer indicates information about the crop, tillage and herbicide regime.

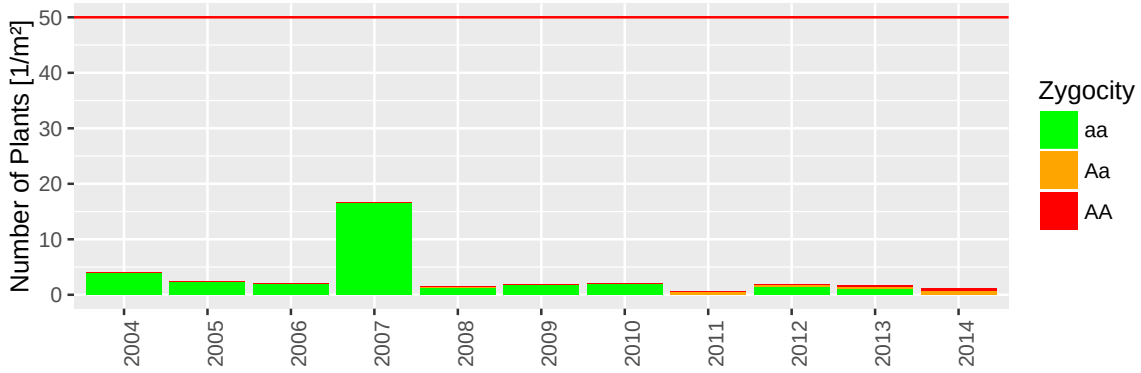
Example Z279: (Atlantis Status S)

Observational Data

FieldID	Year	Infestation	AtlantisCont	MaisterCont	P197X	W574X
Z279	2013	1	97	100		
Z279	2014	0				

Random Forest
Predicted Status: S
Predicted Frequency (R): 0.23

Simulation Model



Field History

Year	Crop	Seeding Date	Tillage	Flufenacet	PRE (Other)	FOP (F)	FOP (S)	DIM (F)	DIM (S)	ALS (F)	ALS (S)	Alt (F)	Alt (S)	Glyphosate
2004	HORVW	normal	Cultivator				X							
2005	TRZAW	late	Cultivator										X	
2006	HORVW	normal	Cultivator										X	
2007	BRSNW	normal	Cultivator		X	X								
2008	TRZAW	normal	Cultivator										X	
2009	HORVW	normal	Cultivator										X	
2010	ZEAMX	normal	Plough								X			
2011	TRZAW	late	Cultivator								X			
2012	ZEAMX	normal	Plough								X			
2013	ZEAMX	normal	Plough								X			
2014	TRZAW	late	Plough								X			

Figure 4.20: Comparison of the predicted resistance status by random forest and simulation model to observational data for field Z279. Field Management by the farmer indicates information about the crop, tillage and herbicide regime.

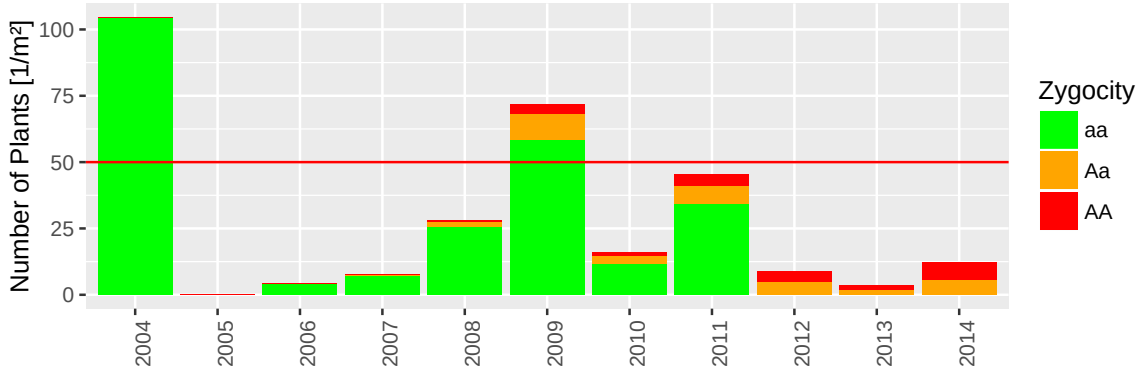
Example Z284: (Atlantis Status R)

Observational Data

FieldID	Year	Infestation	AtlantisCont	MaisterCont	P197X	W574X
Z284	2013	2	64	76		
Z284	2014	1	68	79	6	6

Random Forest
Predicted Status: R
Predicted Frequency (R): 0.86

Simulation Model



Field History

Year	Crop	Seeding Date	Tillage	Flufenacet	PRE (Other)	FOP (F)	FOP (S)	DIM (F)	DIM (S)	ALS (F)	ALS (S)	Alt (F)	Alt (S)	Glyphosate
2004	TRZAW	normal	Disc							X				
2005	TRZAW	normal	Disc								X			
2006	HORVW	normal	Cultivator	X										
2007	BRSNW	normal	Plough		X			X						
2008	TRZAW	normal	Cultivator		X						X			
2009	HORVW	normal	Plough	X		X								
2010	TTLWI	normal	Cultivator		X	X				X	X			
2011	TRZAW	normal	Cultivator								X			
2012	HORVS	normal	Disc				X							
2013	ZEAMX	normal	Disc								X			
2014	TRZAW	late	Plough								X			

Figure 4.21: Comparison of the predicted resistance status by random forest and simulation model to observational data for field Z284. Field Management by the farmer indicates information about the crop, tillage and herbicide regime.

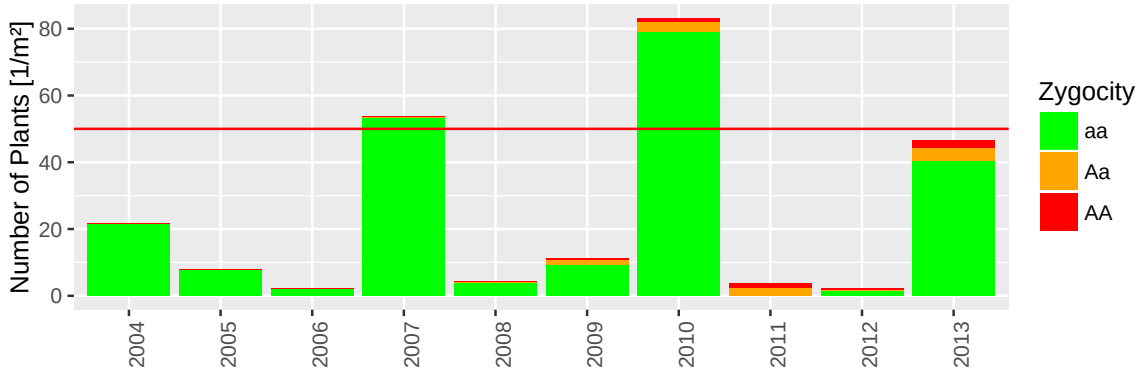
Example Z318: (Atlantis Status S)

Observational Data

FieldID	Year	Infestation	AtlantisCont	MaisterCont	P197X	W574X
Z318	2014	2	100	100	0	0

Random Forest
Predicted Status: S/I
Predicted Frequency (R): 0.44

Simulation Model



Field History

Year	Crop	Seeding Date	Tillage	Flufenacet	PRE (Other)	FOP (F)	FOP (S)	DIM (F)	DIM (S)	ALS (F)	ALS (S)	Alt (F)	Alt (S)	Glyphosate
2004	BRSNS	normal	Cultivator											X
2005	TRZAW	late	Cultivator				X							
2006	HORVW	normal	Disc										X	
2007	ZEAMX	normal	Cultivator								X			
2008	TRZAW	late	Cultivator										X	
2009	HORVW	normal	Cultivator				X							
2010	ZEAMX	normal	Cultivator								X			
2011	TRZAW	late	Cultivator								X			
2012	HORVW	late	Cultivator			X							X	
2013	ZEAMX	normal	Cultivator								X			

Figure 4.22: Comparison of the predicted resistance status by random forest and simulation model to observational data for field Z318. Field Management by the farmer indicates information about the crop, tillage and herbicide regime.

Table 4.17 shows a summary of the comparison of both approaches among the 16 fields. A clear misclassification with the Random Forest approach was observed for H289, where a case was predicted as S but was I. This case was correctly classified by the simulation model. Z116 was another case were the random forest prediction was incorrect while the simulation model predicted the case correctly. On the other hand Z029 was correctly predicted as R by the random forest approach while the simulation model predicted S. Z009 and Z279 were also inaccurately predicted by the simulation model while being correctly classified by the random forest approach. However, a decision rule on which approach to follow in case of unequal predictions needs to be established. The sampling size of 16 is too small to differentiate the samples and identify under which scenario each tool predicts a wrong result. However, no sample was clearly misclassified by both approaches.

Example	Atlantis Status Observed	Result Random Forest	Result Simulation Model
H012	S	S (0.27)	Some resistance observed at low levels
H289	I	S 0.39)	low infestation levels with some resistance observed
H362	R	R(0.75)	resistance established with occasional high infestation
M001	R	R (0.77)	resistance established with occasional high infestation
M007	R	I (0.66)	resistance established with low infestation
M017	R	I/R (0.66)	resistance established with occasional high infestation
M018	R	R (0.77)	resistance established with occasional high infestation
M027	R	I (0.61)	traces of resistance with low infestation levels
Z002	S	S (0.31)	no resistance with low/elevated infestation levels
Z009	S	S (0.21)	traces of resistance with occasional higher infestation
Z023	I	R (0.71)	developing resistance with low/elevated infestation levels
Z029	R	R (0.70)	no resistance with occasional high infestation levels
Z116	S	I/R (0.69)	no resistance with low infestation levels
Z279	S	S (0.23)	traces of resistance with low infestation levels
Z284	R	R (0.86)	resistance with occasional high infestation
Z318	S	S/I (0.44)	traces of resistance with occasional higher infestation

Table 4.17: Prediction Summary for both the Random Forest approach and the simulation model. Values for Random Forest present the predicted groupings and the frequency of a sample being predicted as R. Numbers were rounded to two decimals, while the prediction occurs on seven decimal places, explaining the differences observed for some samples. Interpretation of the model results was derived from plots on page 139 ff

4.4 Discussion

Various studies show the importance of early detection for herbicide resistance (Collavo et al., 2013; Rummland et al., 2014). However, detecting resistance early enough is difficult for the farmer and requires additional analysis in the greenhouse and laboratory. Even with the decreasing costs of resistance detection by laboratory analysis no reliable field test kit is yet available. Therefore it was attempted to predict the resistance situation using a supervised learning algorithm and a simulation model. The benefit for the farmer with these methods is that, once implemented, they are easily usable and low in cost. The outcome of both simulations is very good and items were classified with high precision. While the random forest algorithm was developed using soil data and variables derived from crop management, the simulation model requires a more complex set of input variables. This was achieved by experimenting a lot with these variables within reasonable ranges and setting up a variable set that works reasonably well for the majority of fields tested. As a result of this, the farmer would only need to provide a crop rotation and the relevant soil parameters for the field to be tested. This consequently enables the farmer to run both algorithms with only the provision of these data.

4.4.1 Supervised Learning

The developed resistance algorithm is able to distinguish well between R, I and S (figure 4.4). This shows that the data used to train the algorithm represents enough variability to predict the test data accurately. Although the initial prediction using only R and S showed a number of misclassified items (table 4.10) using the absolute frequencies of prediction as R enabled reasonably successful discrimination between groups. Using the final test dataset of the 36 test cases, no resistance case (R) was predicted as either S or S/I. This scenario would have been a problem as it suggests to the farmer not to change farming practices when facing a severe problem or already observing one. The opposite scenario, a sensitive case S) being classified as I or R occurs in 3/13 samples. This suggests to the farmer that he would need to apply changes while in reality no resistance development is visible yet. While this shows a misclassification and an potential investment by the farmer in IWM it does not create a negative resistance situation for him. With the classified I cases the situation is a bit different. 2/7 cases were either grouped into S or R which creates however only in the first scenario a problem for the farmer as the system fails to detect a necessary change. As a summary the adjustment of the prediction outcome with the extension to more categories gave a valuable improvement

to the classification. However there are still scenarios in which the prediction fails. To further enhance the prediction accuracy the addition of more data points as suggested by Banko and Brill (2001) could help. Furthermore, an extended testing of the model could potentially identify scenarios under which the algorithm fails. Figure 4.3 suggests that crop management data is of higher importance for accurate prediction than soil data. However, as shown in section 3.3.2 both factors are linked with each other. The conclusion is therefore that the crop management differentiates better than soil data but does not represent a measure of importance in terms of resistance development. The approach of using supervised learning algorithms in the field of herbicide resistance research as described in this work is the first application of this tool in this field. Reasons for that are mostly that the available data source is not available, especially field history information. While there are studies, e.g. Bürger et al. (2012), analyzing large field history databases, they were lacking field observations. Another study by Beckie et al. (2008) screened for resistance and analyzed questionnaires to identify causes of resistance. However, the development of a prediction tool was not in the focus of their study.

4.4.2 Simulation Model

The simulation model developed here combines several ideas presented in other works as stated in section 4.2.2. These works were combined to represent the life-cycle of *A. myosuroides* with all its factors as they occur in reality. Newly developed was the extension to represent three resistance patterns, following work done by Böttcher (2003). Furthermore there is no current simulation for *A. myosuroides* that combines various population-describing parameters (e.g. the germination in different cohorts, tillage regimes, crop management, soil parameters) and connects this to a genetic model to simulate resistance development. The complexity of the model was necessary since testing the model with a more simplified simulation did not yield the precision found (table 4.17). The three loci simulated here were developed based on findings from chapter 2 that stated that there are two patterns of resistance to ACCase-inhibitors (FOP/DEN vs. FOP/DEN/DIM). As no differentiation between the two occurring ALS mutations was carried out, their varying incidences could not be explained by the model. However, findings from chapter 3 also delivered no explanations for the different occurrences, leading to the representation of resistance to ALS-inhibitors by one locus. Knight (2016) does not account for this in his simulation, though an implementation of EMR as a monogenic trait was implemented in his model. Testing random sets of combinations enabled the finding of a suitable set to describe the tested cases reasonably well (table 4.17). This does not

necessarily mean that this is the only possible combination of parameters. Compared to Knight (2016) soil seedbank estimates and germination rates were considerably larger but within data reported in the literature. This parameterization gave a descent fit with observations for fields that have been sampled over multiple years. However, the small size of only 1ha simulated is small and was used to achieve rational computing speeds with the stochastic component implemented. The stochastic component is necessary to provide some variation for parameters which were for the most part estimated. One critical point was the consideration of an established resistance to ACCase-inhibitors in the model. Here Knight (2016) assumes 1% of the seeds in the soil seedbank for his simulation. Based on the established resistance to ACCase-inhibitors and interviews with the farmer this was believed to be too low and so the values were adapted to 30% for resistance to FOP/DENs and 10% to the DIMs in the initial seedbank. The initial seedbank is difficult to determine and is therefore estimated for most of the models e.g. Knight (2016). Rummland (2015) developed one model with actual measurement data of the soil seedbank. Here, literature estimates were used. Since many parameters were estimated, a true validation of the model in other areas and under different scenarios is necessary. Compared to the previously mentioned RIM model, the simulation presented here is based on a genetic model to predict the influence of herbicide resistance on infestation levels. This is an important difference as the constant evolution of resistance with an increase in infestation levels can be modeled on a very fine scale with no general assumptions about herbicide control overall. However, the underlying efficacies of the genotypes used in this study were also estimated and not experimentally defined. A fairly new approach was used in describing the soil characteristics. Dunker et al. (2002) assessed different soil factors in a previous simulation, but in the present study, a connection of the findings from chapter 3 was attempted. Actual coverage of different soil clusters as they were obtained by analyzing soil maps were implemented into the CA. This, together with the field history information, allows an exact description of the field situation with the CA. The model therefore requires knowledge of a high number of parameters. Renton et al. (2014) points out that a greater level of detail makes the interpretation of the results more difficult as the estimation of the impact of an individual parameter cannot be accurately assessed. This was however not in the focus of the development since the highest priority was given to the accuracy of the simulation compared to observational data.

So far the trends of the model describe the observed situation reasonably well. This is considered as a first validation, though wider testing in other areas will also be necessary for the tool to be implemented at a broader scale.

Furthermore, new insights into the time course of resistance development have been gained. As Bagavathiannan et al. (2014) showed in his simulation for two loci, a failure to stop using a given herbicide soon after the occurrence of resistance increases the speed of resistance to the second one. The authors related that to the increase in seedbank size. Here, further evidence is shown as table 4.15 and figure 4.5 suggest that in years without the selection by an ALS-inhibitor the number of seeds carrying a resistant gene to ALS-inhibitors increases. This is a result of an established resistance pattern as it was found for the ACCase-inhibitors here. Mutations, which are assumed for herbicide resistance to occur randomly (Jasieniuk et al., 1996) are therefore likely to involve plants being already resistant to another MoA as is the case here. Spraying an ACCase-inhibitor in this case increases the number of genotypes resistant to ACCase-inhibitors but potentially carries a resistance allele to another MoA accelerating therefore the build up of a multiple resistant population. This explains why under certain circumstances the reported resistance cases occurred after few years of application. The pattern occurs in particular if the soil has the capabilities to hold high populations and seedbank sizes. The comparison between Z038 4.5 and Z023 (p.133) shows this clearly. Both fields were farmed similarly by the same farmer between 2004 and 2012. In the first case the resistance is well established, yet in the second it is not very evident in the field but clearly detectable at the greenhouse and laboratory level. This is simulated well given that traces of resistance are predicted for Z023 by the simulation, with an established resistance found for Z038. In this case the model (and the random forest algorithm) clearly show this undergoing selection. While Neve et al. (2011); Bagavathiannan et al. (2014) use a threshold to define if a case is resistant or not, here the outcome for every year was compared to the observation obtained for that field. This demands increased precision, while giving deeper insights into the contribution of each year to resistance development.

Furthermore, the simulation shows that soils with a higher carrying capacity serve like a reservoir in the field, allowing patches with higher abundances of plants in the field. Resistant plants occurring in patches were also found by Balgheim (2009) observing that patches of resistant and sensitive plants exist within a field. While the sampling at the field level does not account for this, it is important for the dynamics occurring within the field. Cavan et al. (1998) showed in their analysis that resistant and sensitive plants from a single patch are more related to each other than resistant plants from two different patches of the same field. This indicates that some further subdivision within the field is taking place. The assessment of the underlying soils, as seen in chapter 3, is therefore important to categorize and estimate the total number of seeds within a field.

Furthermore, this allows for a more robust estimation of the initial number of resistant plants based on a given mutation rate.

4.4.3 Comparing the overall prediction accuracy

Overall prediction accuracy of the 16 samples characterized by both approaches was very high. All three previously defined resistance categories (R, I, S) were also found in the test dataset. This is important as it allows assumptions to be made about the prediction accuracy for both sensitive and resistant fields. There was only one example (Z318, table 4.17) in which both tools predicted an elevated risk of resistance while in reality no resistance was yet observed. However, this is generally still acceptable as both tools predict only minimal development of resistance, indicating that the risk is rather low. A problem that occurs is that of how to judge samples in which both tools give different outcomes with one being right and the other being wrong. Here, further test data needs to be screened to assess the scenarios under which a given tool fails. One possible solution to this could also be to test seed or leaf material in the laboratory and greenhouse for verification of the true status. Essentially, it highlights the benefits of having both approaches as this minimizes the samples that could not be classified accurately. It means that if two approaches work completely independently from one other and come to the same conclusion, the overall conclusion is very reliable. To achieve the establishment of a prediction tool for use by farmers, this is very valuable as accuracy is crucial. Both approaches involve computational costs to varying degrees. While the prediction via random forest needs <1sec one simulation run needs about 1min. This problem needs to be overcome if the tool set is to be implemented more widely.

4.4.4 Limitations of the prediction and outlook

At the moment there are still a few limitations for both the random forest algorithm and the simulation model that need to be overcome.

Random Forest Prediction

- As we find only biotypes that are resistant to ALS-inhibitors based on target-site mutations, the algorithm was trained on an area selecting with this type of resistance. As the application is for all *A. myosuroides* areas the algorithm needs to be verified in an area with EMR as well. If the algorithm were to predict those

cases correctly, the conclusion could be that the general farming practices leading to resistance are not too different than the target-site dominated area of Hohenlohe. This would imply that either the type of resistance finally selected occurs randomly (as chapter 2 shows for the two different TSR mutations) or that the selection for a given resistance mechanism occurred before the time covered in this study (10 years). However, EMR is thought to occur mainly in areas spraying sublethal doses as research by Neve and Powles (2005a) shows. It will be interesting to see whether these cases are correctly classified, as sublethal doses were not considered in the algorithm so far.

- Following up on the previous point a broadening of the study and extension to other areas in Germany or other countries might be necessary to validate the tool. This will show whether the conclusions related to the field history of this study may be generalized.

Model

- Only dispersal via seeds between cells of the CA is covered by the simulation. Pollen has yet to be considered. This will need to be implemented, as pollen transfers typically involve greater distances than seeds (Colbach and Sache, 2001). This is however partially accounted for as one cell is simulated as comprising 100sqm. This simulates pollen clouds reaching many plants.

- Currently only a small overall area is simulated, totaling 1ha. Larger values were not considered with regard to computing speed but will need to be tested in future versions of the simulation.

- For the moment a very general approach is used to describe herbicides. The group POST_Alt in particular refers to any herbicide being applied that was not covered by any other group. The average survival was assumed to be 0.2 which does not distinguish well between the different MoA applied. Here the addition of categories might need to be considered.

- The model considers only target-site resistance of three independent loci. Richter et al. (2014) developed a model that also enables the consideration of metabolic resistance. This needs to be considered since metabolic resistance to ALS-inhibitor plays a role in other areas as found by (Knight, 2016).

- The model does not include fitness penalties as reported e.g. by Menchari et al. (2007a). A reduction of seed production, for instance, as something crucial for propagation of a trait, has yet to be implemented in the current version.

- Differences in seed decay as found by Zwerger (1993) are currently not implemented in the model. This might be an important point to consider especially with regard to different tillage regimes. Future work will need to address this point to better compare different tillage systems.

- Among the factors described in Chapter 1 several were not considered in the simulation. Weather data indicating the crop stand in particular years was not considered in the simulation, and so fixed data for the crop density were considered in every year. Severe weather effects or variation in the crop, resulting in an annual varying in infestation of *A. myosuroides* are not currently implemented.

Outlook and future work

Despite the points above, a complete linkage of both algorithm and simulation model needs to be carried out. The accuracy of the two together is very high, since even in the case of a false classification of one, the other is correct. However, as previously stated a solid evaluation of cases being wrongly classified by one tool and not another needs to be performed. Both tools (in conjunction or separately) can also be used to identify risk areas in Germany and elsewhere. While the focus here was mostly the application in a single field, the practice can be broadened to entire regions by assessing similar soil types with regionally relevant crop rotations to consider their risk of developing resistance. This early detection system could be interesting for both farmers and companies in identifying areas that are potentially prone to resistance. Finally, an easy to use interface for the farmer needs to be developed. The interface should provide an easy to use layout where data of field management and soil data are easily entered.

Chapter 5

Conclusions and Perspectives

5.1 Conclusions

Resistance to herbicides is regarded as a common threat in agriculture today. *A. myosuroides* as a key weed in cereal based crop rotations has developed multiple resistances to important herbicides within the ACCase- and ALS-Inhibitor family. ALOMY has therefore been for many years a weed in the focus of herbicide resistance research with regards to prevalence of resistance and its genetic background (Menchari et al., 2006; Délye et al., 2010b; Knight, 2016). The present study differentiates itself from other studies in several different respects. One important difference is the random selection of fields. While the three initial starting points were chosen based on confirmed resistance cases, the subsequent sampling of surrounding fields is regarded as random sampling. The majority of fields therefore showed infestation at below agronomically relevant levels. This is important as it implies that in these fields either the *A. myosuroides* infestation is low or the efficacy of the herbicide used is still very high. As Collavo et al. (2013); Rummland (2015) showed, accumulated levels of resistance persist in the absence of a fitness penalty and so determining the early phases of resistance development is crucial and is regarded as the most important phase (Neve et al., 2009). These low frequencies are difficult to detect even with greenhouse or laboratory analyses. An attempt to predict those on the field level was therefore presented in this work. To set the basis for such a tool a characterization of the underlying dynamics of herbicide resistance in space and time and the identification of factors contributing to herbicide resistance was done. The number of fields that were sampled for this purpose (1225) greatly exceeds the size found in other studies, enabling the analysis of close to all fields over several km^2. This is in contrast to other studies covering greater geographical ranges with fewer observations (e.g. Menchari et al. (2006); Knight (2016)). The number of observations greatly enhances the statistical power of the study as the level of detail included to describe a given location is very high. It provides furthermore greater reliability in the findings of genetic diversity, resistance development and spatial distribution because it enables one to compare different management scenarios and soil aspects in close proximity. The approach of studying numerous fields with *A. myosuroides* according to their resistance status and combining the findings with field history and soil information is the first of its kind. The focus was not only on confirmed resistance cases but also on cases with low, or in some years, no *A. myosuroides* infestation. This provides a true estimation of the prevalence of resistance in a certain geographical region. Most studies on herbicide resistance, including this one, try to assess the resistance situation indirectly by assessing the resistance status of field survivors after herbicide application by the farmer. On exception to that is the study

by Rummland (2015) for *Apera spica-venti* assessing the soil seedbank before and after application in a field trial. The correct classification of early resistance cases from single seed samples might therefore be difficult to achieve. This consequently leads to the requirement of multiple observations from different years in fields where resistance is not yet suspected. In the absence of clear resistance patterns which were not found in chapter 2 a rating based on classes (R, I and I) was therefore developed. Problems encountered include the often low infestation at harvest in the field with all survivors after the herbicide application by the farmer resistant to the herbicides of interest tested. These cases represent a considerable enrichment of the soil seedbank but are not directly visible for the farmer in the field. To detect cases which suffer from resistance early enough, tools for an accurate prediction of the resistance situation within the field were developed and tested in this study.

Key findings ordered by the topics addressed in the different chapters were:

1. Chapter 2 focused on the abundance of resistance at a local scale. It was found that the prevalence of resistance to ACCase-inhibitors was very pronounced while it was under development for the ALS-inhibitors in the study area. Only very few fields showed both high infestation levels after herbicide application by the farmer and low efficacies to the ALS-inhibitors in greenhouse samples combined from these field survivors. The temporal dynamics were analyzed by looking at variation in the same fields over time and with different crops. The similarity in neighboring fields was assessed to analyze spatial dynamics and test if exchange of resistance alleles between neighboring fields occurs. Local occurrence of resistance in the absence of isolation by distance, together with a high temporal variability caused by different crops selecting for different patterns of resistance was observed. This resulted in similar temporal patterns found only for those fields with the same status to both modes of action tested.

2. Chapter 3 analyzes key factors on their impact of resistance development. In addition to management data that is commonly assessed to answer these questions, soil data was evaluated. The findings were used to explain differences in the phenotype observed in chapter 2. The data suggested that soils with a higher carrying capacity for ALOMY are at a higher risk of resistance development. Furthermore, a greater abundance of fields classified as R were found among those with a lower diversity in management of *A. myosuroides* .

3. Chapter 4 focused on the development of resistance prediction tools with chapter 2

and chapter 3 serving as the basis. Using the "Random Forest" supervised learning technique, a new tool for the prediction of resistance was tested. The successful development of a simulation model used together with the random forest algorithm was also developed. Both of these predicted the selected test cases reasonably well, with 15/16 cases correctly classified. However, this is not yet a true validation as the extent to which these tools can be generalized for other areas still needs to be determined. The focus is therefore on the development of a tool that 1) accurately predicts resistance development at the field level and 2) is easily used by the farmer with the information they have available.

The random sampling design of fields revealed several new mutations that were not previously reported for *A. myosuroides* (e.g. I1781V, I1781T) implying that there is a considerable standing genetic variation in the two loci observed (Neve et al., 2009). Evidence for a directed selection by some herbicides (Focus Ultra selecting for I1781X and MaisTer fluessig selecting for W574X) was also presented, indicating that the selection outcomes can be influenced by the farmer and assessments of samples without knowledge of the management regime can be misleading. Overall, the infestation at the field level was found to be low for the majority of samples, while local hotspots were found. The problem of comparing the situation in different crops to each other was overcome by normalizing values by the mean and standard deviation for all fields with the same crop sampled in the same year. This is one way of achieving reasonable comparisons but requires large numbers of samples for accurate assessment. The hotspots of high infestation found were mostly attributed to pronounced resistance. Resistance to FOPs/DENs in particular was well established in almost all fields. Farmers were mostly aware of this and used other modes of action in combination with these products to achieve reasonable control in the field, explaining why the overall infestation was at low levels. The detected hotspots were attributed by some farmers with the underlying soils but their individual analysis revealed no direct link with the infestation levels. While some farmers observed high infestation values on soils permitting a higher carrying capacity, others had the infestation still under control. Higher carrying capacities of some soils with regard to *A. myosuroides* plants are important in the development of herbicide resistance as their characteristics allow higher population sizes. In addition, the unfavorable growing conditions for cereals – mainly all expressions of waterlogging – that can occur on these soils create a competitive advantage to ALOMY. The higher plasticity of the weed allows it to grow well even if conditions for the crop are unfavorable, however. The often observed occurrence of multiple soils per field makes achieving adequate tillage more challenging for the farmer, which again

favors *A. myosuroides* . This is important for farmers to know since higher inputs for the successful control and resistance management of *A. myosuroides* are needed at locations that are prone to resistance development.

A fluctuating resistance pattern over time, considering both MoA, was also found. Besides some fields with very pronounced resistance, the analysis of the temporal aspects revealed no temporal patterns within fields. This makes sampling with regard to resistance development sensitive to the time when an observation is taken and differs from approaches analyzing existing resistance. Future work on assessing the resistance situation for a weed where resistance is suspected but not confirmed would therefore require sampling a field at least twice in preferably different crops. This is different to studying confirmed resistance cases, as done by e.g. Délye et al. (2010a). These authors found that samples from confirmed resistance cases did not differ much over time and can therefore be used in comparisons with other studies. A similar conclusion can be drawn from this study based on the analysis of resistance alleles to FOPs/DENs seen in Chapter 2. The inclusion of the observations for ALS-inhibitors resulted in a very variable pattern over time, indicating that in the developmental phase of resistance to a given MoA samples from different years are very variable.

Given that no isolation by distance was seen and no pattern was identified by clustering, a classification of fields into R, I and S was necessary. These categories were derived based on the analysis across sample patterns observed and their relation to the situation from the farmer's perspective. While patterns across these samples were detectable, multiple combinations of these patterns were observed for the same field with samples from different years leading to the grouping presented here. The grouping is therefore a generalization of the observed situation in the field. The sampling size of 106 fields is too small to deal with a significantly larger number of groups in both, explaining resistance causes and prediction models. The analysis of the samples provided a broader insight into factors previously discussed and related to resistance (Lutman et al., 2013; Beckie et al., 2008; Knight, 2016). The strong connection of factors related to non-chemical weed control and their positive effect on resistance prevention was shown. These factors include for one, the crop rotation. Summer crops, as stated by Lutman et al. (2013) had a very strong effect in reducing the *A. myosuroides* population. Lower numbers of *A. myosuroides* will ultimately result in a reduced risk of resistance occurrence through a lower frequency of resistance genes in the field (Jasieniuk et al., 1996; Powles and Yu, 2010). The same applies for delayed seeding dates and ploughing. However, it was shown that delayed seeding was attributed primarily to corn farming, since the delayed harvest

of corn prevents the early seeding of winter wheat. Diversity in herbicide MoA was not found to be a main factor influencing resistance development. Resistant fields had a higher treatment frequency with ALS-inhibitors compared to intermediate and sensitive samples. Furthermore, intermediate fields had a lower treatment frequency with herbicides in general, but saw broadly the same frequency of ALS-inhibitors used. Independent interpretation of these factors can only be carried out to a limited extent. Therefore a PCA was performed with both soil and management data. Reduced reliance on IWM and a replacement with simplified crop rotations and high use of ALS-inhibitors was therefore the main impact on resistance development. There is more of a gradient than a clear cut difference between the groups identified in the study. While some groups are comparable in their management, the underlying soil differentiates them between higher and lower frequencies of resistance. Soils with higher risk of resistance were found to have higher clay contents and high field capacities in combinations with low levels of water penetrating to deeper layers of soils. This makes them prone to water logging which *A. myosuroides* can deal with better than the crop, resulting in greater population sizes. For farmers, these results are important as they suggest that resistance development can be prevented by applying adequate measures. Furthermore a differentiated view across fields is necessary to identify those with a higher risk (carrying capacity) to favor resistance development. These are mostly fields which show the highest present density of *A. myosuroides* and those where appropriate tillage and seeding is the most difficult. Under similar management, resistance will most likely evolve there first. Survivors of herbicide application should therefore be seen critically, and counteractive measures in the form of IWM applied. Simplified winter cereal-based crop rotations with an over-reliance on ALS-inhibitors for weed control alone poses a risk aside from the locations, since it permits high population sizes given that *A. myosuroides* is well-adapted to winter cereals. Meanwhile certain crop rotations, especially corn farming, carry a very low risk of the development of resistant *A. myosuroides* to ALS-inhibitors although their reliance on this MoA is high. It was possible to relate the status of resistance to a given management regime and soils but it was not possible to relate these findings to a given resistance mutation (P197X, W574X). This indicates once more that even if some herbicides clearly select for some mutations resistance is selected from the standing genetic variation (the mutations naturally occurring in the population). Transfer of the obtained results into tools for direct use by consultants or farmers was attempted in Chapter 4. The discrimination of the three resistance categories as done in Chapter 3 compared with the findings of individually developing resistance in Chapter 2 set the basis for this. The

first attempt to use a supervised learning algorithm (Random Forest) for resistance prediction was presented here. A second approach, using a simulation model, was already developed by several authors but so far lacks either applicability, accuracy or field validation (Colbach et al., 2006; Knight, 2016). Results of both the simulation and the random forest approach lead to a high accuracy in forecasting, either of the correct event or the correct tendency of resistance. No resistant sample was grouped as sensitive by both tools, which would be the most problematic result since it would suggest that the farmer not react to an upcoming threat or existing resistance. Another possibility is that, a sensitive sample may be classified as intermediate. In terms of the outreach of the prediction this is wrong, but it does not negatively impact the farmer as it implies actions that are not disadvantageous but which further decrease the chance of resistance development. It needs to be stated here that the data used for the validation of both tools was not used in their development. The tools presented here provide a first step in the development of a resistance prediction tool on the field level. Further validation is still required, mainly with populations from other regions. The good results in the simulation studies in this study might be attributed to the high level of TSR in the samples. This contrasts with findings from Knight (2016) who had high percentages of EMR and tested the simulation on fields with suspected resistance, preventing the comparison between R and S samples. A validation of his model could therefore not be done. Compared to another popular model, RIM, the model developed here bases the prediction on the increase of resistant genotypes presented by a genetic component in the simulation. This enables greater precision as the reduced efficacy of herbicides is connected to the presence of genotypes conferring resistance, and not on estimates of the lower efficacy of a given herbicide. However, this requires greater precision in the estimations of parameters, as discussed earlier.

The simulation model by itself provided further interesting insights into resistance development as it predicts that a small enrichment with resistance genes can be greatly enriched by pronounced resistance to another MoA that lacks efficient control. This results from the propagation of resistance genes on genotypes that possess resistance to multiple MoA, a phenomenon already found in simulations by Bagavathiannan et al. (2014). An established resistance to ACCase-inhibitors was found in this study therefore setting the requirements for this phenomenon to occur with regard to resistance to ALS-inhibitors . Furthermore, it was seen in the simulation that soils with a higher carrying capacity act as reservoirs of (resistant) seeds and therefore need special attention from the farmer. This is an interesting result and is in agreement with observations by Bal-

gheim (2009) who reported resistant populations occurring in patches in the population. Applications of these results are interesting in terms of discussions over precision farming, though these are beyond the scope of this study. They are also interesting in relation to the identification of areas with higher risk for resistance development on a larger scale.

In general software provides an easy and cost efficient way to distribute tools to farmers and advisers using them in their recommendations. The accurate prediction that was achieved by combining two separate methods and comparing them side by side provides a novel approach, though it has yet to be discovered the scenarios in which individual approaches fail. Overall the study gave valuable insights into both the abundance and the temporal and spatial variability of resistance, identifying resistance development occurring locally within a field. Simplified crop rotations were identified as factors strongly contributing to resistance development. Different management patterns were identified with each abundance of R, I and S. The combined analysis of soil properties together with the applied management revealed that soils which allow high carrying capacities of *A. myosuroides* explain the difference in the resistance status under similar management. The prediction of these resistance statuses was possible with the two approaches tested. Each approach applied to an individual sample was able to balance out a possible false classification from the other with high agreement amongst the two approaches combined. This sets a strong basis for the prediction of resistance development at the field level and enables the farmer to apply counteractive measures before observing problems in the control of *A. myosuroides* in the field. The outreach of the tool needs to be verified by sampling in other areas but promising results for weed resistance management were obtained with the data presented.

5.2 Future Work

Several open points of the work remain to be answered in the future.

It was shown that resistance development typically occurs individually within a field. This was in agreement with some studies but contradicted findings by Délye et al. (2010a) who found genetic variation to be similar between "source" and "sink" fields. Furthermore it was shown that different management regimes in combination with different soils result in different resistance statuses. This study could not determine whether all fields had the same genetic material of *A. myosuroides* at the beginning of a herbicidal selection. It remains unknown whether this has an influence on the initial composition and frequency of resistance genes within the population occurring in a field. The analysis

of the population structure could therefore answer the question of whether the selection through crop management, together with the underlying soil, is the only explanation for the differences observed, or whether differences occur across populations that need to be considered in addition to the already mentioned factors. It would also serve to clarify the kinship of populations occurring together at the local field level, though it will not determine whether the assumed mutation rate can be considered equal in all fields, nor the extent to which a difference in the assumed mutation rate confers changes in the resistance development within a field. The latter would need to be addressed with simulation models such as the one described in this work.

Since the study was carried out in an area dominated by TSR the results on the causes of herbicide resistance need to be validated in an area with EMR being present exclusively or in combination with TSR such as in the UK (Knight, 2016). Data will show whether the findings are generally applicable. Given that low doses were found to be a strong selector for EMR this factor would need to be included in the analysis as well (Neve and Powles, 2005b). Yu and Powles (2014) however pointed out that low doses generally select for both EMR and TSR. Therefore, this point might be of lesser importance than the factors assessed here which demonstrate the greater impacts of the non-chemical measures.

Many of the limitations that need to be addressed with the prediction of herbicide resistance have been already addressed (Chapter 4) and are therefore only summarized here. As noted, for findings of field history the two approaches chosen to predict resistance need further validation in areas with EMR. In addition the option to consider EMR as a possible resistance mechanism needs to be implemented in the simulation model. The basis for this was established by Richter et al. (2014).

Besides the prediction of individual field data, both simulation approaches can also be used to predict resistance risk on a regional scale. Sufficient information on the underlying soils to predict the clusters derived in chapter 3 are necessary for that. One could therefore use the methods presented in chapter 4 to identify risky strategies in given locations or identify risky areas in unknown locations. This can be regarded as a proactive prediction of possible upcoming resistance problems.

The approaches used for the prediction of herbicide resistance provide a very good basis for further work on refining the tools for other weeds. The prediction tools presented here were based on 106 fields for which both multiple field observations and field management observations were available. For a defined area, such as the study area presented here, this provides a solid prediction and would therefore reflect the minimum number of

fields required to extend this to other weeds within a similarly heterogeneous environment with diverse management patterns. Given that only 30% of the contacted farmers were providing field management data, the required number of fields would greatly increase. For weeds other than *A. myosuroides* and compareable and for areas with different farming systems these numbers can greatly vary.

Reliable information on this from literature is not currently available. While a representative sample size is sufficient for the random forest algorithm to be trained, adapting the simulation model for other weed species requires additional work. While related species, such as *Apera spica-venti*, that occur under similar cropping regimes might only need an adaptation of the parameters, unrelated species will need a deeper understanding of the biology of the species, requiring additional work to adopt the simulation.

Chapter 6

Summary

A. myosuroides is a key weed in many agricultural areas of Western Europe. While the control of *A. myosuroides* by both ACCase-inhibitor and ALS-inhibitor was sufficient in the past, cases of suspected and confirmed resistance are increasing, representing a threat to agriculture. A case control study including various scales was conducted to assess the resistance situation at a local landscape level. The aim of the study was to 1) properly assess the resistance status at a given location, 2) characterize the spread and temporal development of resistance, 3) analyze factors contributing to resistance and 4) attempt to predict resistance development at the field level to properly manage resistance evolution before the occurrence of a problem.

1225 fields were sampled for between one and six years. The infestation level in the field was estimated and the remaining efficacy of seeds from field survivors on ACCase and ALS-inhibitors were tested using whole plant greenhouse bioassays. Additional laboratory analysis was carried out to discover the underlying diversity of resistance patterns. The infestation level at harvest was low in the majority of fields, indicating that either the level of *A. myosuroides* infestation was low or the efficacy of the applied herbicide by the farmer was high. At harvest time only 6% of all the samples collected showed infestation levels reducing yields, while 70% of the fields showed no *A. myosuroides* infestation or only individual *A. myosuroides* plants remaining. Analyzing the remaining efficacy of herbicides on these field survivors showed that resistance to the ACCase-inhibitors of the FOP and DEN class was found in >83% of the samples and is therefore considered to be established in the study area. Resistance to ALS-inhibitors was found to remain under development with <32% of the samples showing low efficacy. This indicates that there are only few fields suffering from high infestation levels of *A. myosuroides* together with low efficacy to ACCase and ALS-inhibitors . Among the resistance mechanisms, all previously reported target-site mutations for both modes of action were found. No relationship was found by relating the genetic diversity of the resistance alleles occurring at the ACCase locus to the geographical distance. The genetic difference was found to be lower across multiple fields from the same farmer and was lowest for multiple subsets of the same field over several years compared to the overall variation. Assessing both MoA together with greenhouse and field data revealed no grouping structure that could be extracted for a large range of fields. Both results suggest that the resistance structure of each population is field specific.

The different observations for each field were translated into resistant samples (R), intermediate samples (I) and sensitive samples (S) with regard to ALS-inhibitors . Intensive interviews with the farmers were carried out to obtain management data from recent

years. In addition a soil map containing relevant parameters influencing the biology of *A. myosuroides* was analyzed. Evaluating the data shows that fields with higher diversity in *A. myosuroides* management through the use of integrated weed management options are less likely to show resistance. Soils with higher carrying capacity of *A. myosuroides* however play an important role in differentiating R from S cases under similar management. Therefore all factors that aim to obtain low population sizes (management) of *A. myosuroides* or the factors which cause them (lower carrying capacity of the soil) prevent the development of resistance to ALS-inhibitors .

To predict the outcomes at the field level two approaches – a simulation model and a supervised learning algorithm (Random Forest) – were trained and validated with the obtained data. In 15 out of 16 cases, both were able to predict the correct resistance status and both separately showed some false classification with 12% and 19% for the random forest algorithm and the simulation model respectively.

The study highlights that resistance development can involve management by the farmer, though the diversification of *A. myosuroides* management. It also shows that the risk of resistance development is higher for fields that are more favorable for *A. myosuroides* . The prediction tools presented in this work are a first step in the development of software that enables the farmer to accurately predict the risk at the field level, allowing for early counteractive measures in cases of high resistance risk. Further validation of these tools with more data from other areas is necessary to assess the extent to which they can be generalized.

Chapter 7

Appendix

7.1 Soil Map Legend

Tab. 1: Übersicht Datenfelder

FELD	INHALT	FELD	INHALT
A Beschreibung der verbreitet auftreten Böden		PH_LN	pH unter LN (Stufe)
BGL	Bodengroßlandschaft	PH_WALD	pH unter Wald (Stufe)
KE	Bodenkundliche Kartiereinheit	KLZ	Klassenzeichen der Bodenschätzung
KE_KULEG	Kartenkurzlegende	BEGL_BOD	Begleitböden
KE_COL	Farbsymbol der Kartiereinheit	SONSTIGE	Sonstige Angaben
KE_SIG	Übersignatur der Kartiereinheit	**B Physiko-chemische Bodenkennwerte**	
NUTZUNG	Nutzung	FK	FK (Stufe)
RELIEF	Relief	FK_MIN	FK minimal bis 10 dm Tiefe (mm bzw. l/m^2)
BOD_T	Bodentyp (Klartext)	FK_MAX	FK maximal bis 10 dm Tiefe (mm bzw. l/m^2)
BOD_C	Bodentyp (Code)	NFK	nFK (Stufe)
MAT_T	Ausgangsmaterial der Bodenbildung (Klartext)	NFK_MIN	nFK minimal bis 10 dm Tiefe (mm bzw. l/m^2)
MAT_1	Ausgangsmaterial der 1. Schicht (Code)	NFK_MAX	nFK maximal bis 10 dm Tiefe (mm bzw. l/m^2)
MAT_2	Ausgangsmaterial der 2. Schicht (Code)	LK	LK (Stufe)
MAT_3	Ausgangsmaterial der 3. Schicht (Code)	KF	kf-Wert (Stufe)
MAT_UG1	Untergrenze Ausgangsmaterial der 1. Schicht (dm u. Fl)	KAK	KAK (Stufe)
MAT_UG2	Untergrenze Ausgangsmaterial der 2. Schicht (dm u. Fl.)	KAK_MIN	KAK minimal bis 10 dm Tiefe (mol_c/m^2)
BODA_1	Bodenarten der 1. Schicht (Kürzel KA5)	KAK_MAX	KAK maximal bis 10 dm Tiefe (mol_c/m^2)
BODA_2	Bodenarten der 2. Schicht (Kürzel KA5)	K_FAKTOR	K-Faktor (Stufe)
BODA_3	Bodenarten der 3. Schicht (Kürzel KA5)	**C Bodenfunktionen nach „Bodenschutz 23“**	
BODA_UG1	Untergrenze Bodenarten der 1. Schicht (dm u. Fl.)	NATVEG	Sonderstandort für naturnahe Vegetation (Stufe), (Bodenschutz 23)
BODA_UG2	Untergrenze Bodenarten der 2. Schicht (dm u. Fl.)	NATBOD	Natürliche Bodenfruchtbarkeit (Stufe), (Bodenschutz 23)
KALK_T	Karbonatführung (Klartext)	AKIWAS_LN	Ausgleichskörper im Wasserkreislauf unter LN (Stufe), (Bodenschutz 23)
KALK_C	Karbonatführung (Code)	AKIWAS_W	Ausgleichskörper im Wasserkreislauf unter Wald (Stufe), (Bodenschutz 23)
GRUND	Gründigkeit (Stufe)	FIPU_LN	Filter und Puffer für Schadstoffe unter Wald (Stufe), (Bodenschutz 23)
DUWUBA	Durchwurzelbarkeit (Stufe)	FIPU_W	Filter und Puffer fürSchadstoffe unter LN (Stufe), (Bodenschutz 23)
WAHUFORM	Waldhumusform (Code)	GESBEW_LN	Gesamtbewertung unter LN (Stufe), (Bodenschutz 23)
HUMUS_OB	Humusgehalt im Oberboden (Stufe)	NATVEG	Sonderstandort für naturnahe Vegetation (Stufe), (Bodenschutz 23)
HUMUS_UB	Humusgehalt im Unterboden (Stufe)	GESBEW_W	Gesamtbewertung unter Wald (Stufe), (Bodenschutz 23)

Figure 7.1: Soil class characteristics and their explanation for the soil classes extracted from the map obtained from Reg (2015). Original document in german.

7.2 Maps of ACCase- and ALS-inhibitor distribution among years

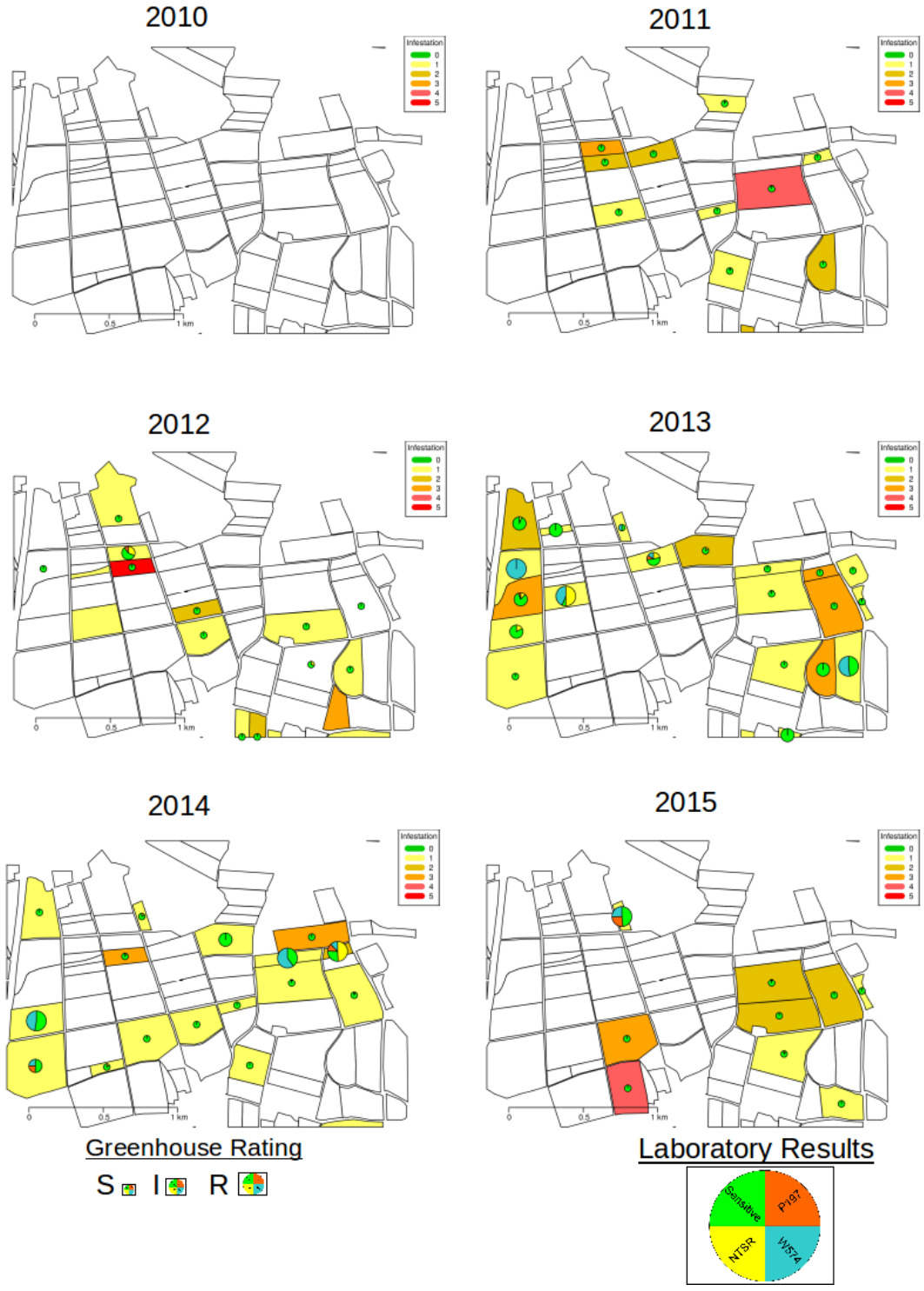


Figure 7.2: ALS resistance development for Location H between 2010 and 2015 for the fields assessed. The maps comprise three layers of information. The field color indicates the infestation level according to the scale provided. The size of the pie shows the efficacy of Atlantis WG 500g/ha in the greenhouse (legend: bottom left). The slices of the pie indicate the portion of the mutations (legend: bottom right)

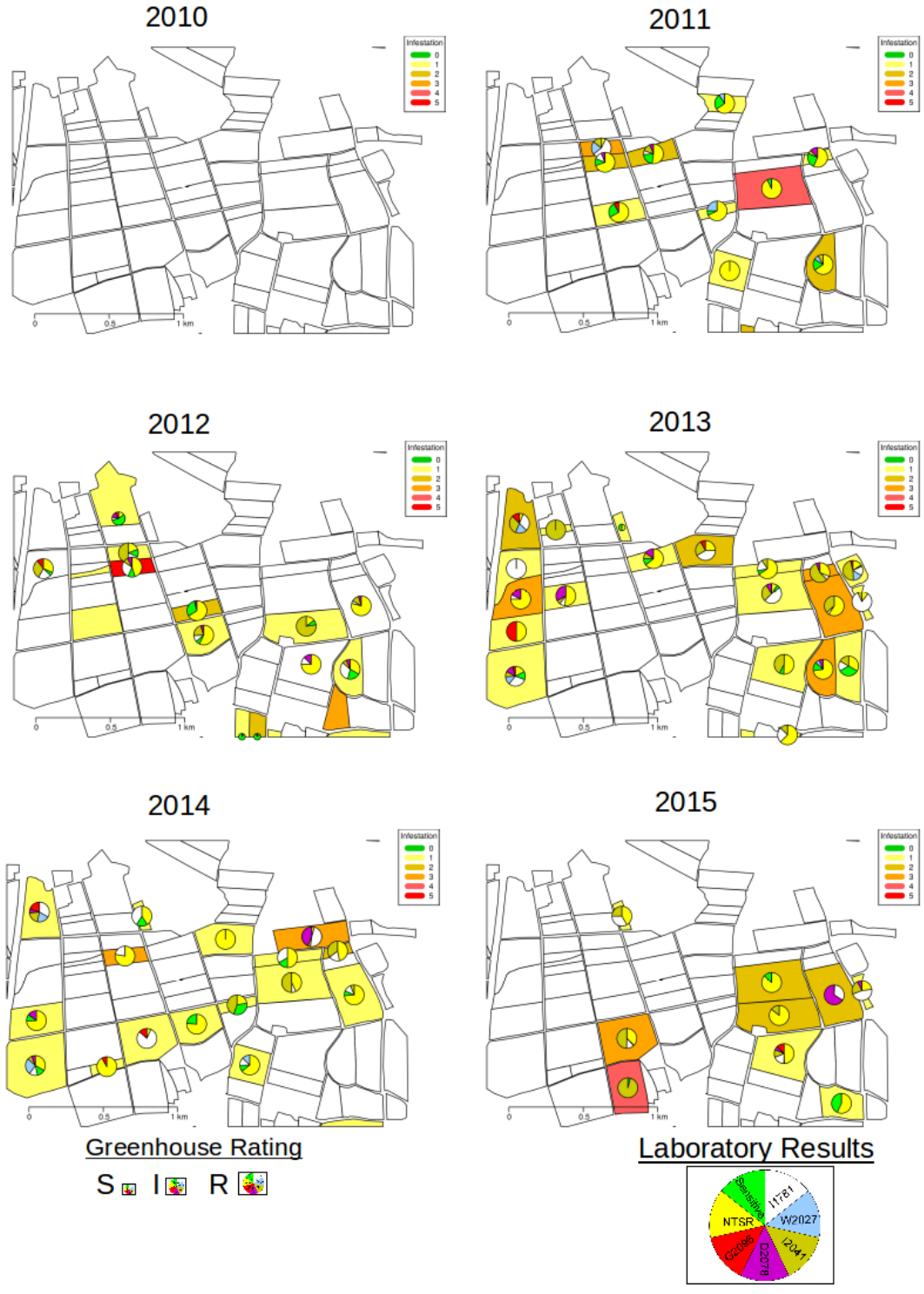


Figure 7.3: ACCase resistance development for Location H between 2010 and 2015 for the fields assessed. The maps comprise three layers of information. The field color indicates the infestation level according to the scale provided. The size of the pie shows the efficacy of Atlantis WG 500g/ha in the greenhouse (legend: bottom left). The slices of the pie indicate the portion of the mutations (legend: bottom right)

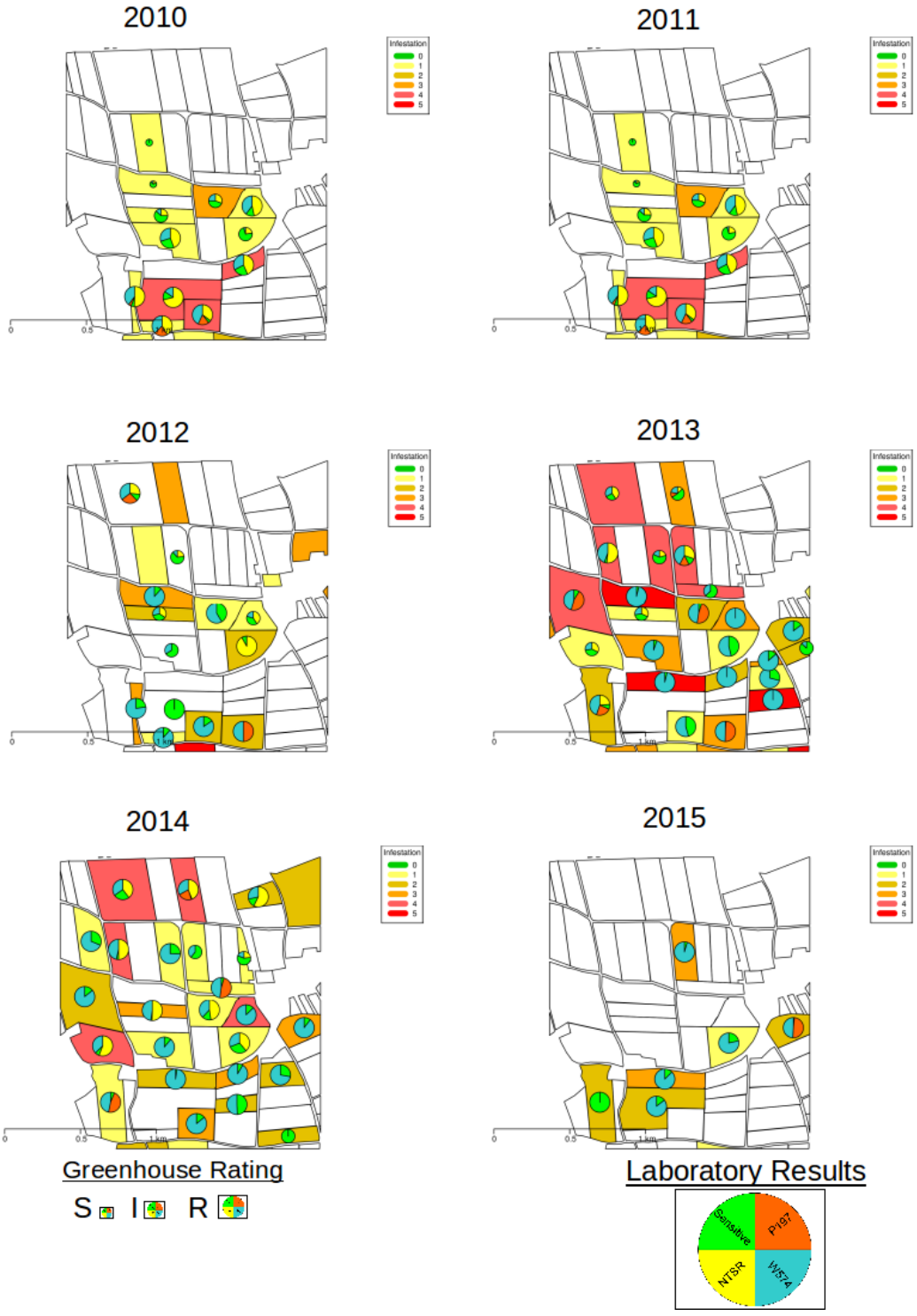


Figure 7.4: ALS resistance development for Location M between 2010 and 2015 for the fields assessed. The maps comprise three layers of information. The field color indicates the infestation level according to the scale provided. The size of the pie shows the efficacy of Atlantis WG 500g/ha in the greenhouse (legend: bottom left). The slices of the pie indicate the portion of the mutations (legend: bottom right)

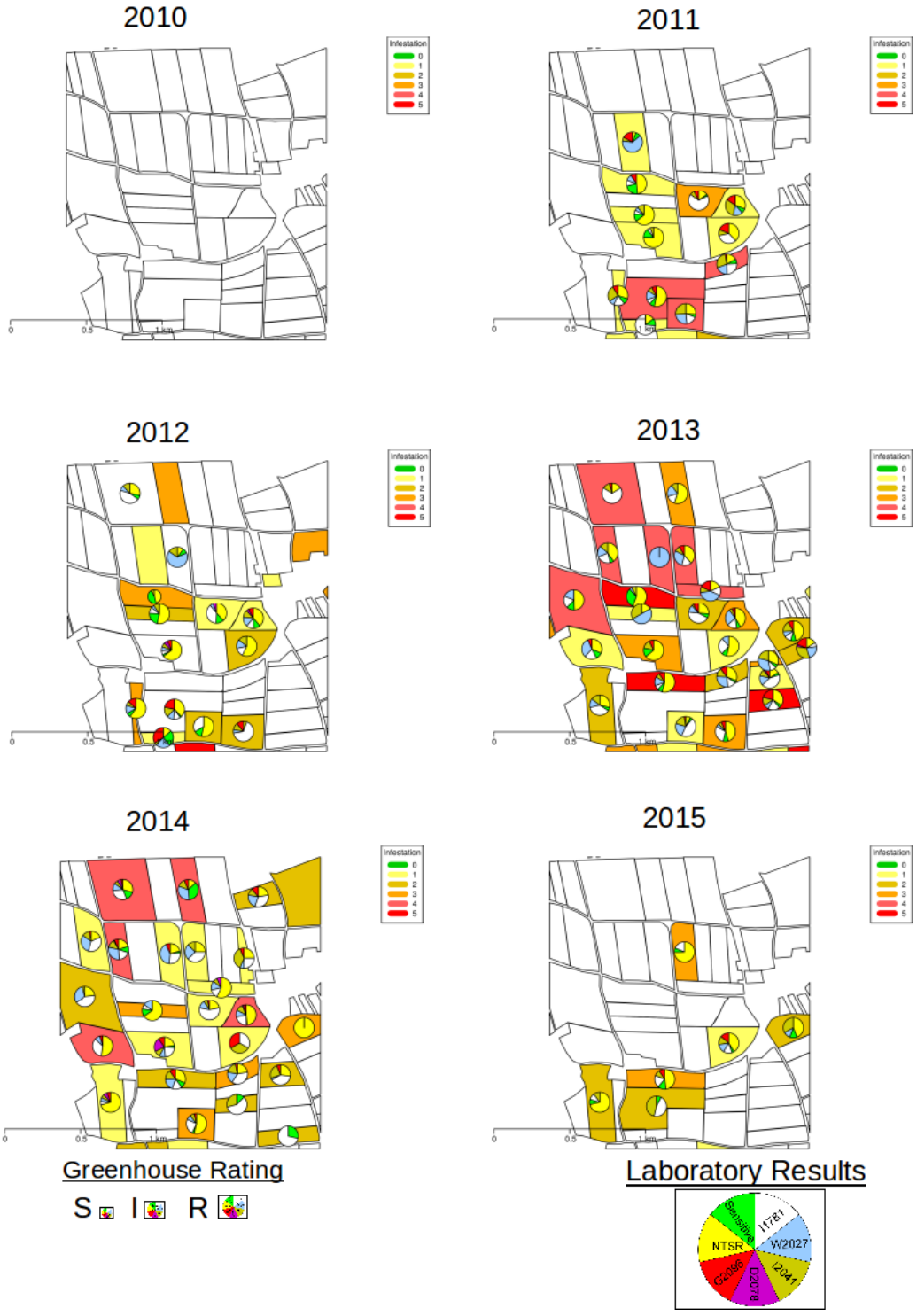


Figure 7.5: ACCase resistance development for Location M between 2010 and 2015 for the fields assessed. The maps comprise three layers of information. The field color indicates the infestation level according to the scale provided. The size of the pie shows the efficacy of Atlantis WG 500g/ha in the greenhouse (legend: bottom left). The slices of the pie indicate the portion of the mutations (legend: bottom right)

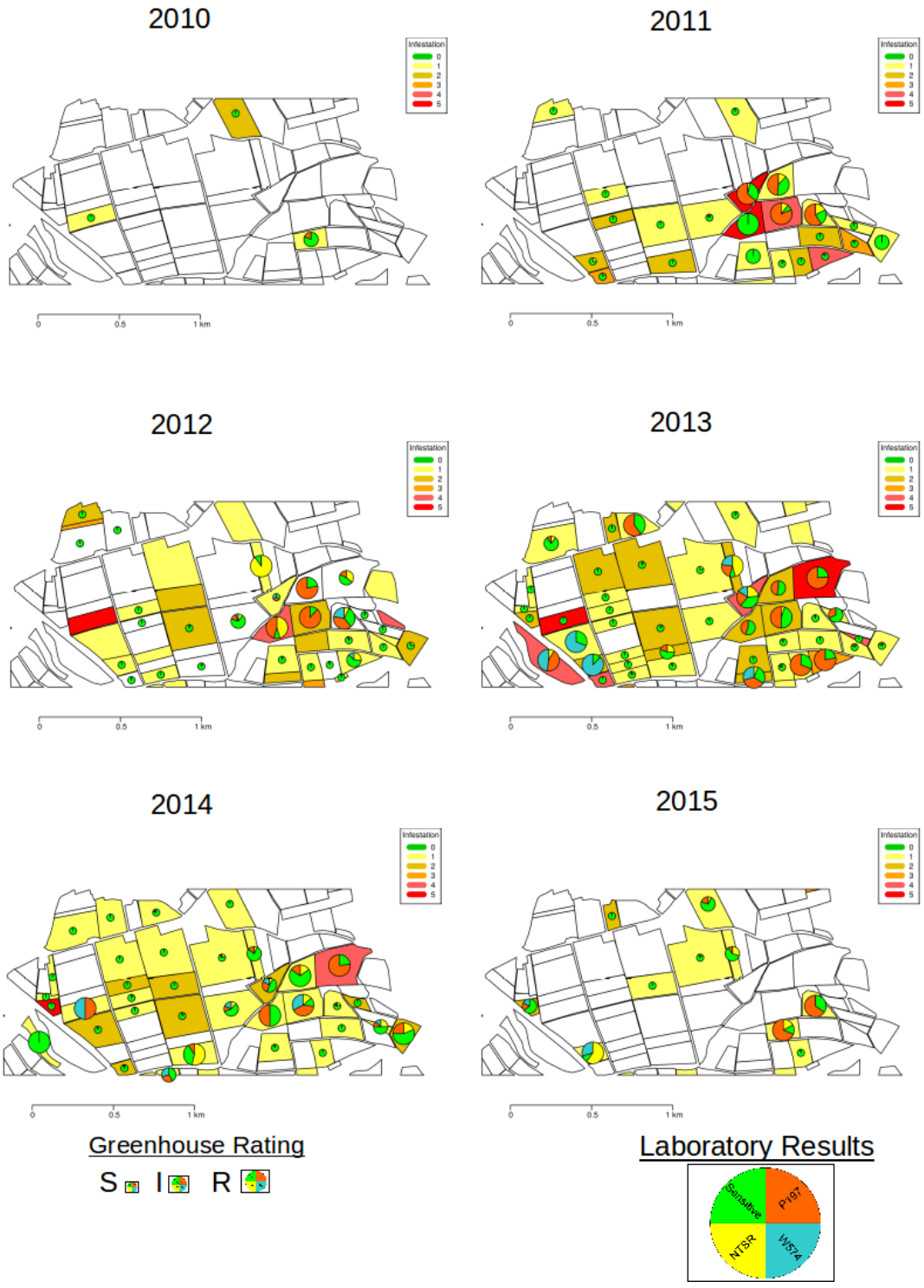


Figure 7.6: ALS resistance development for Location Z between 2010 and 2015 for the fields assessed. The maps comprise three layers of information. The field color indicates the infestation level according to the scale provided. The size of the pie shows the efficacy of Atlantis WG 500g/ha in the greenhouse (legend: bottom left). The slices of the pie indicate the portion of the mutations (legend: bottom right)

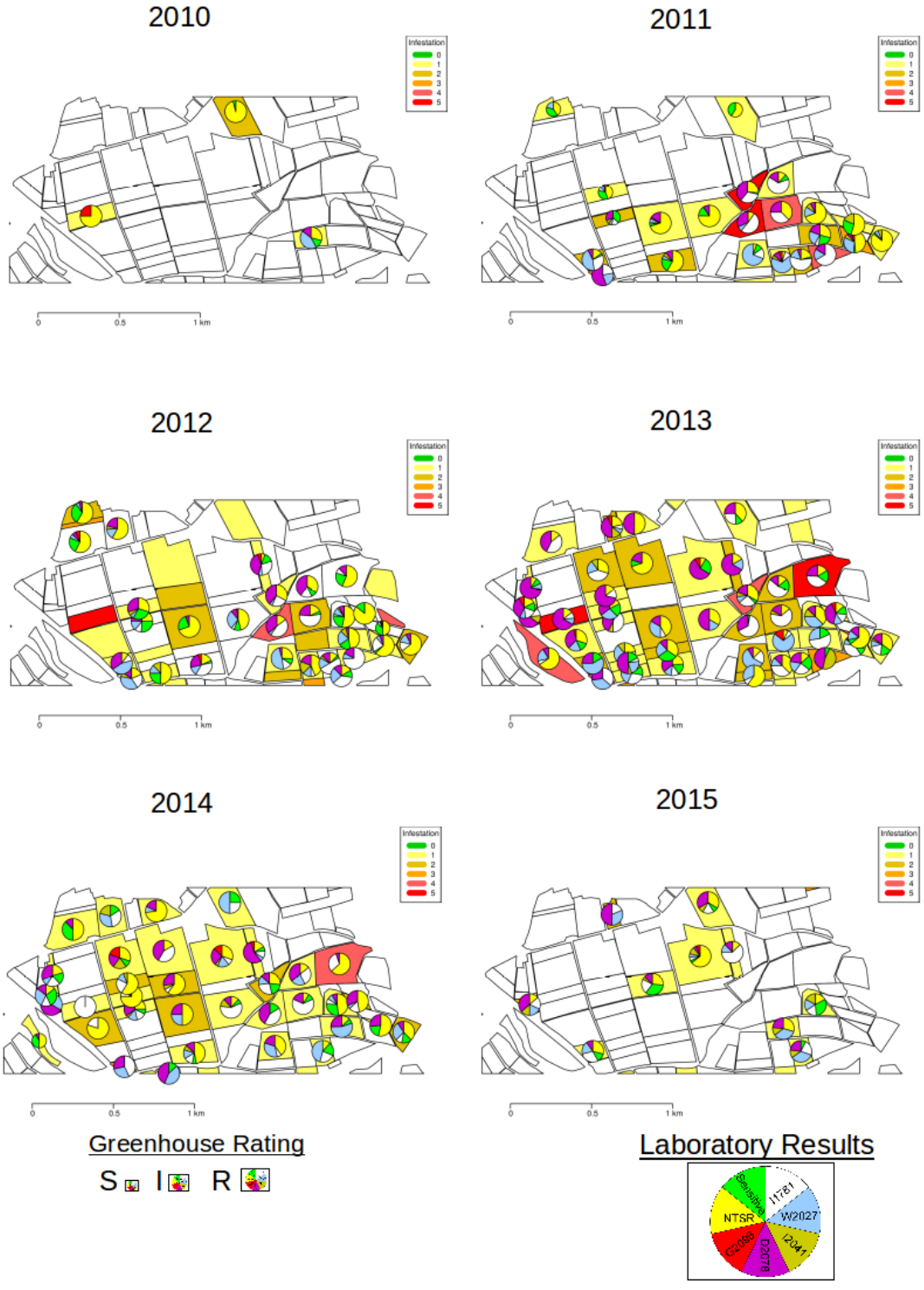


Figure 7.7: ACCase resistance development for Location Z between 2010 and 2015 for the fields assessed. The maps comprise three layers of information. The field color indicates the infestation level according to the scale provided. The size of the pie shows the efficacy of Atlantis WG 500g/ha in the greenhouse (legend: bottom left). The slices of the pie indicate the portion of the mutations (legend: bottom right)

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